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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53(c).

Docket Number	00786/433001	Type a plus sign (+) inside this box --->	+

INVENTOR(S)/APPLICANT(S)

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TITLE OF THE INVENTION (280 characters max)

REPAIRING OR REPLACING TISSUES OR ORGANS

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification	Number of pages: 47	☒ Claims	Number of pages: 8
☒ Drawings	Number of sheets: 16	☒ Abstract	Number of pages: 1

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ A check or money order is enclosed to cover the filing fees. ☒ The Commissioner is hereby authorized to charge filing fees not covered and apply any credits to Deposit Account Number: 03-2095	FILING FEE AMOUNT	$80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government. The names of the U.S. Government agency and the Government contract numbers are NIH PO1-CA-80124-02 and NIH R24-CA-85140-01.

☒ Applicant claims small entity status under 37 C.F.R. § 1.27.

Respectfully submitted,

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DATE: 31 October 2002
REGISTRATION NO.: 35,238

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PROVISIONAL APPLICATION

UNDER 37 C.F.R. § 1.53(c)

APPLICANT : Rakesh K. Jain and Dai Fukumura

TITLE : Repairing or Replacing Tissues or Organs

PATENT
ATTORNEY DOCKET NO: 00786/433001

REPAIRING OR REPLACING TISSUES OR ORGANS

Statement as to Federally Sponsored Research

This work was supported in part by the National Institutes of Health (PO1-CA-80124-02 and R24-CA-85140-01). The government may have certain rights to this invention.

Background of the Invention

In general, the invention features methods and compositions for repairing or replacing damaged or diseased tissues or organs.

Approximately 8 million surgical procedures are performed annually to treat tissue loss or end-stage organ failure. Although these therapies have saved lives, they have severe limitations. The need for organ transplants exceeds the supply of available organs. In 2000, over 50,000 people on the transplantation waiting list failed to receive transplanted organs. Thus, alternatives to traditional transplantation therapies are needed.

Transplantable engineered tissues could be used to address chronic organ shortages if technical limitations could be overcome. The development of clinically transplantable three-dimensional engineered tissues is limited by the fact that tissue assemblies greater than 100-200 μm require a perfused vascular bed to supply nutrients and to remove waste products, metabolic intermediates, and secreted products. Mature functional vascular networks have been difficult to engineer given that vascular development is a complex event involving various cell types and many different growth factors.

During embryonic development, endothelial cells form tubes and connect to form the primary capillary plexus, this process is termed angiogenesis. New vessels are formed by splitting existing vessels in two, or by sprouting from existing vessels. This

primary network is remodeled and pruned in a process termed vessel maturation to form a distinct microcirculatory units that include capillaries, arteries and veins. VEGF is a growth factor that functions to induce endothelial cells proliferation and sprouting. The nascent vasculature recruits mesodermal cell, such as pericytes and endothelial cells, to surround the developing vasculature; and angiopoietin-1 (Ang-1) is a growth factor that plays an important role in recruiting and regulating the assembly of non-endothelial vessel wall components. The interaction between endothelial cells and pericytes leads to the reorganization of vessels into arteries, arterioles, capillaries, venules, or veins. TGF-β, a growth factor that inhibits endothelial cell proliferation and migration, induces mesodermal cell differentiation, and stabilizes the mature capillary network.

Summary of the Invention

The present invention features methods and compositions for promoting blood vessel formation or engineering blood vessels in damaged, diseased, or transplanted organs, and for producing functional microvascular networks useful in tissue engineering.

In one such aspect, the invention provides a method for promoting blood vessel formation (e.g., angiogenesis, vasculogenesis, formation of an immature blood vessel network, blood vessel remodeling, blood vessel stabilization, blood vessel maturation, blood vessel differentiation, or establishment of a functional blood vessel network) or engineering blood vessels in a mammal (e.g., a human). The method involves administering one or more cells including, but not limited to, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, fibroblasts, adipocytes having or not having a genetic modification, preadipocytes, or stem cells that differentiate into one of these cell types to a tissue or organ of a mammal in need of increased blood vessel formation or engineered blood vessels (e.g., a mammal with a damaged or diseased tissue or organ, or a mammal requiring a transplant). In one preferred embodiment, the mammal has a deficiency of a particular cell type. In another preferred embodiment, the mammal has damage to a tissue or organ, and the method

provides a dose of cells sufficient to increase a biological function of the tissue or organ by at least 5%, 10%, 25%, 50%, 75%, 90%, 100%, or 200%, or even by as much as 300%, 400%, or 500%. In yet another preferred embodiment, the mammal has a disease, disorder, or condition, and the method provides a dose of cells sufficient to treat or stabilize the disease, disorder, or condition. For example, the mammal may have a disease, disorder, or condition that results in the loss, atrophy, dysfunction, or death of cells. Exemplary treated conditions include a neural, glial, or muscle degenerative disorder, muscular atrophy or dystrophy, heart disease such as congenital heart failure, hepatitis or cirrhosis of the liver, an autoimmune disorder, diabetes, cancer, a congenital defect that results in the absence of a tissue or organ, or a disease, disorder, or condition that requires the removal of a tissue or organ, ischemic diseases such as angina pectoris, myocardial infarction and ischemic limb, accidental tissue defect or damage such as fracture or wound. In other embodiments, the mammal has an increased risk of developing a disease, disorder, or condition that is delayed or prevented by the method.

In a related aspect, the invention features a method for promoting blood vessel formation or engineering blood vessels in a tissue or organ (e.g., a human tissue or organ) by administering preadipocytes, adipocytes having or not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, or fibroblasts to a tissue or organ in need of increased blood vessel formation or an engineered blood vessel network. The tissue or organ may be *in vivo* or *ex vivo*. In various preferred embodiments, the tissue or organ is selected from the group consisting of bladder, brain, nervous tissue, glia, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and cartilage. In other preferred embodiments, the administered cells are from the same mammal. In other embodiments, the tissue or organ is from a different mammal than the administered cells. Preferably, the method further includes administering the tissue or organ to a recipient mammal. The tissue or organ

may be from a donor of the same species as the recipient or from a different species (e.g., pig or primate). Preferably, the administration of cells increases the biological function of a diseased or damaged tissue or organ by at least 5%, 10%, 25%, 50%, 75%, 100%, 200%, or even by as much as 300%, 400%, or 500%.

In one preferred embodiment of any of the above aspects, the method further involves administering to the tissue or organ one or more cells selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, stem cells that differentiate into endothelial cells, endothelial cell lines, or endothelial cells generated from stem cells *in vitro*. Preferably, the method further involves administering a matrix to the tissue, organ, or mammal. A matrix may be composed of any biocompatible material, such as synthetic polymers or hydrogels. For some applications, biodegradable materials are particularly desirable.

In other preferred embodiments, the method increases the number of cells of the tissue or organ by at least 5%, 10%, 20%, more desirably by at least 25%, 30%, 35%, 40%, 50%, 60%, or even by as much as 70%, 80%, 90 or 100% compared to a corresponding tissue or organ. In preferred embodiments, the method increases the biological activity of the tissue or organ by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, or even by as much as 200%, 300%, 400%, or 500% compared to a corresponding, naturally-occurring tissue or organ.

In other preferred embodiments of the above aspects, the method increases blood vessel formation (e.g., angiogenesis, vasculogenesis, formation of an immature blood vessel network, blood vessel remodeling, blood vessel stabilization, blood vessel maturation, blood vessel differentiation, or establishment of a functional blood vessel network) in the tissue or organ by at least 5%, 10%, 20%, 25%, 30%, 40%, or 50%, 60%, 70%, 80%, 90%, or even by as much as 100%, 150%, or 200% compared to a corresponding, naturally-occurring tissue or organ. In other preferred embodiments, the tissue or organ is selected from the group consisting of bladder, brain, nervous tissue, glia, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver,

lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and cartilage. In another aspect, the invention provides a method for transplanting a tissue or organ in a mammal (e.g., a human). The method involves administering to the mammal a tissue or organ having at least 5%, 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, 400%, or 500% more perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, such as embryonic stem cells, mesenchymal cells, or preadipocyte, than a corresponding naturally-occurring tissue or organ. In one preferred embodiment, the method further involves administering to the mammal one or more blood vascular endothelial cells, lymph vascular endothelial cells, or endothelial cell lines, freshly prepared primary culture endothelial cells (e.g., cells obtained from the donor or recipient mammal), or endothelial cells generated from stem cells *in vitro*. The administered cells may be from the recipient mammal or from another mammal. In other preferred embodiments of the above aspects, the cells are part of a microvascular scaffold or a perfused microvascular scaffold.

In one preferred embodiment, the method further involves administering to the mammal a matrix. A matrix may be composed of any biocompatible material, such as synthetic polymers or hydrogels. Preferred matrices are biodegradable.

The invention also provides methods to form a microvascular scaffold by incubating at least two cell types (e.g., endothelial cells or endothelial precursor cells and cells of at least one other cell type) under conditions that produce a network of blood vessels. Preferably, the blood vessels grow throughout a cultured tissue. The microvascular scaffolds can be administered to mammals to repair or replace an endogenous tissue or organ.

In one such aspect, the invention provides a method for producing a microvascular scaffold. The method involves culturing (i) a first cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, and endothelial cell lines; and (ii) a second cell selected from the group consisting of perivascular cells,

vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, preadipocytes, adipocytes, and fibroblasts, under conditions that allow formation of a microvascular scaffold. In one preferred embodiment, the first and second cells are cultured in the presence of a matrix. In another preferred embodiment, the cells are grown on the matrix. In another preferred embodiment, the matrix encapsulates the cells. Optionally, the method further involves administering a matrix to a mammal. In one preferred embodiment, cells are present in the matrix prior to, during, or after the matrix is administered to the mammal.

The matrix may be composed of any biocompatible material (e.g., synthetic polymers or hydrogels). For some applications, biodegradable materials are particularly desirable. Preferably, the microvascular scaffold is a stable network of blood vessels that endures for at least 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 3 months, 6 months, or even as long as 12 months. In one preferred embodiment, the microvascular scaffold is a perfused scaffold that is integrated into the circulatory system of the tissue, organ, or mammal. In another preferred embodiment, the microvascular scaffold is a mature network of differentiated vessels that includes arterioles and venules.

In a related aspect, the invention features a microvascular scaffold that includes (i) a first cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, and endothelial cell lines; and (ii) a second cell selected from the group consisting of perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, adipocytes, and fibroblasts. In one embodiment, the microvascular scaffold persists for at least 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 3 months, 6 months, or even, most preferably, as long as 12 months.

In a another related aspect, the invention features a perfused microvascular scaffold that includes (i) a first cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, and endothelial cell lines; and (ii) a second cell selected from the group consisting of mesenchymal precursor cells (e.g., embryonic stem cells or 10T1/2 cells) and mesenchymal cells.

In preferred embodiments of the above aspects, the microvascular scaffold further includes a matrix. In one preferred embodiment, the matrix includes a bioactive molecule (e.g., activin A, adrenomedullin, aFGF, ANF, angiogenin, angiopoietin-1, angiopoietin-2, angiopoietin-3, angiopoietin-4, angiostatin, angiotropin, angiotensin-2, AtT20-ECGF, betacellulin, bFGF, B61, bFGF inducing activity, CAM-RF, ChDI, CLAF, Cox-2, ECDGF (endothelial cell-derived growth factor), ECG, ECI, EDM, EGF, EMAP, endothelins, endostatin, endothelial cell growth inhibitor, endothelial cell-viability maintaining factor, ephrins, Epo, HGF, TNF-alpha, TGF-beta, PD-ECGF, PDGF, IGF, IL8, growth hormone, fibrin fragment E, FGF-5, Factor X, HB-EGF, HBNF, HGF, HUAF, heart derived inhibitor of vascular cell proliferation, IFN-gamma, IL1, IGF-2 IFN-gamma, K-FGF, LIF, leiomyoma-derived growth factor, MCP-1, macrophage-derived growth factor, monocyte-derived growth factor, MD-ECI, MECIF, MMPs, neurothelin, nitric oxide donors, oncostatin M, PD-ECGF, PAI-2, PD-ECGF, PF4, PlGF, prolactin, prostacyclin, protein S, smooth muscle cell-derived growth factor, smooth muscle cell-derived migration factor, tachykinins, TGF-beta, TIMPs, TNF-alpha, TNF-beta, transferrin, thrombospondin, urokinase, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF, VEGI, PF4, 16 kDa fragment of prolactin, prostaglandins E1 and E2, steroids, heparin, 1-butyryl glycerol (monobutyrin), or nicotinic amide). In other preferred embodiments, the matrix includes a chemotherapeutic agent or immunomodulatory molecule. Such agents and molecules are known to the skilled artisan.

In other preferred embodiments of the above aspects, the interstices of the scaffold include one or more additional cell-types including, but not limited to, skin cells, liver cells, heart cells, kidney cells, pancreatic cells, lung cells, bladder cells, stomach cells, intestinal cells, cells of the urogenital tract, breast cells, skeletal muscle cells, skin cells, bone cells, cartilage cells, keratinocytes, hepatocytes, gastro-intestinal cells, epithelial cells, endothelial cells, mammary cells, skeletal muscle cells, smooth muscle cells, parenchymal cells, osteoclasts, or chondrocytes. These cell-types may be introduced

prior to, during, or after microvascular scaffold formation. This introduction may take place *in vitro* or *in vivo*. When the cells are introduced *in vivo*, the introduction may be at the site of the microvascular scaffold or at a site removed from the microvascular scaffold. Exemplary routes of administration of the cells include injection and surgical implantation.

In another aspect, the invention features a tissue or organ that contains a microvascular scaffold and one or more additional cell-types, including; but not limited to, cells derived from bladder, brain, nervous tissue, esophagus, fallopian tube, glia, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and or cartilage.

In another aspect, the invention provides for a method of repairing a diseased or damaged tissue or organ. The method involves implanting one or more cells selected from the group consisting of perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, preadipocytes, adipocytes, and fibroblasts. In one embodiment, the implanted cells increase blood vessel formation (e.g., angiogenesis, vasculogenesis, formation of an immature blood vessel network, blood vessel remodeling, blood vessel stabilization, blood vessel maturation, blood vessel differentiation, or establishment of a functional blood vessel network) or increase the function of a blood vessel network by at least 5%, 10%, 20%, 30%, 50%, 60%, 75%, 80%, 90%, or even by as much as 100%, 150%, or 200% in the damaged tissue or organ as compared to a naturally-occurring, corresponding tissue or organ. In a preferred embodiment, the implanted cells improve the biological function of the diseased or damaged organ by at least 5%, 10%, 20%, 30%, 50%, 60%, 75%, 80%, 90%, 100%, 200%, or even by as much as 300%, 400%, or 500% compared to a naturally-occurring, corresponding tissue or organ. In another preferred embodiment, the implanted cells increase cell number in the diseased or damaged organ by at least 5%, 10%, 20%, 30%,

50%, 60%, 75%, 80%, 90%, or 95% as compared to a naturally-occurring, corresponding tissue or organ.

In a related aspect, the invention features a tissue or organ having at least 5%, 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, 400%, or 500% more of an implanted cell-type selected from the group consisting of perivascular cells vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, preadipocytes, adipocytes, and fibroblasts, than a corresponding naturally-occurring tissue, or organ. In one preferred embodiment, the tissue or organ further contains a transplanted cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, and endothelial cell lines. In another preferred embodiment, the tissue or organ has at least a 5%, 10%, or 20%, 30%, 40%, 50%, 60%, 75%, 80%, 90%, 100%, 200%, or even 300%, 400, or 500% increase in blood vessel formation (e.g., angiogenesis, vasculogenesis, formation of an immature blood vessel network, blood vessel remodeling, blood vessel stabilization, blood vessel maturation, blood vessel differentiation, or establishment of a functional blood vessel network), after implantation of the cell-type compared to a corresponding naturally-occurring control tissue. In another preferred embodiment, the tissue or organ has at least a 5%, 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90% or 95% increase in cell number compared to a corresponding control tissue or organ. In another embodiment, the tissue or organ further comprises cells derived from the group consisting of bladder, brain, nervous tissue, glial tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and cartilage. In another embodiment, the tissue or organ has at least a 5%, 10%, 20%, 30%, 40%, 50%, 70%, 80%, 90%, 100%, 150%, 200%, or even a 300%, 400%, or 500%, increase in biological function compared to a corresponding, naturally-occurring tissue or organ. In one embodiment, the biological function of the tissue or organ is digestion, excretion of waste, secretion, electrical activity, muscle activity, hormone production, or other

metabolic activity. Methods for assaying the biological function of virtually any organ are routine, and are known to the skilled artisan (e.g., Guyton et al., Textbook of Medical Physiology, Tenth edition, W.B. Saunders Co., 2000).

In another aspect, the invention features an organ that includes a tissue of the invention. Optionally, the organ is an engineered organ comprising a microvascular scaffold. In various embodiments, the organ is a bladder, brain, nervous tissue, glial tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, or cartilage. In preferred embodiments, the tissue includes one or more cell-types derived from bladder, brain, nervous tissue, glial tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, or cartilage. Optionally, blood vessel formation, biological function, or cell number of an engineered organ comprising a microvascular scaffold is compared to engineered organs not comprising a microvascular scaffold.

In other embodiments of the preceding aspects, a cell of the invention is transformed with a heterologous nucleic acid. In some embodiments, the administered cells (e.g., adipocytes) are not genetically modified. In other embodiments, the cells are genetically modified to express a bioactive molecule, or heterologous protein or to overexpress an endogenous protein, such as an angiogenesis-related factor selected from the group consisting of activin A, adrenomedullin, aFGF, ANF, angiogenin, angiopoietin-1, angiopoietin-2, angiopoietin-3, angiopoietin-4, angiostatin, angiotropin, angiotensin-2, AtT20-ECGF, Betacellulin, bFGF, B61, bFGF inducing activity, CAM-RF, ChDI, CLAF, Cox-2, ECDGF (endothelial cell-derived growth factor), ECG, ECI, EDM, EGF, EMAP, endothelins, endostatin, endothelial cell growth inhibitor, endothelial cell-viability maintaining factor, ephrins, Epo, HGF, TNF-alpha, TGF-beta, PD-ECGF, PDGF, IGF, IL8, and growth hormone, fibrin fragment E, FGF-5, Factor X, HB-EGF,

HBNF, HGF, HUAF, heart derived inhibitor of vascular cell proliferation, IFN-gamma, IL1, IGF-2 IFN-gamma, K-FGF, LIF, leiomyoma-derived growth factor, MCP-1, macrophage-derived growth factor, monocyte-derived growth factor, MD-ECI, MECIF, MMPs, neurothelin, nitric oxide, oncostatin M, PD-ECGF, PAI-2, PD-ECGF, PF4, PIGF, prolactin, prostacyclin, protein S, smooth muscle cell-derived growth factor, Smooth muscle cell-derived migration factor, tachykinins, TGF-beta, TIMPs, TNF-alpha, TNF-beta, transferrin, thrombospondin, urokinase, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF, VEGI, VEGI, PF4, 16 kDa fragment of prolactin, prostaglandins E1 and E2, steroids, heparin, 1-butyryl glycerol (monobutyrin), and nicotinic amide.

In preferred embodiments of any of the preceding aspects, the cells administered include, but are not limited to, perivascular cell (e.g., pericytes), vascular smooth muscle cells, mesenchymal precursor cells, such as embryonic stem cells, 10T1/2 cells, mesenchymal cells, preadipocytes (e.g., TA1, 3T3-L1, 3T3-F442A, or Ob17), adipocytes, murine embryonic fibroblasts, fibroblast cell lines (e.g., NIH 3T3, Swiss 3T3, BalbC 3T3), and tumor activated stromal cells (e.g., GFP-positive cells isolated from tumors grown in VEGF-GFP mice, EF1a-GFP mice, or Tie2-GFP mice).

In other preferred embodiments of any of the preceding aspects, blood vascular endothelial cells or lymph vascular endothelial cells are derived from freshly prepared primary culture endothelial cells (obtained from the patient who is to receive the graft or from a donor), stem cells that differentiate into endothelial cells, or endothelial cells generated from stem cells *in vitro*. Sources of embryonic stem cells include, but are not limited to, bone marrow derived stem cells or cord blood derived cells. Additional sources include HUVEC, lymphatic endothelial cells, embryonic stem cells , and endothelial pregenitor cells .

In other preferred embodiments of any of the preceding aspects, the matrix may include a collagen gel, a polyvinyl alcohol sponge, a poly(D,L-lactide-co-glycolide) fiber matrix, a polyglactin fiber, a calcium alginate gel, a polyglycolic acid mesh, polyester (e.g., poly-(L-lactic acid) or a polyanhydride), a polysaccharide (e.g. alginate),

polyphosphazene, or polyacrylate, or a polyethylene oxide-polypropylene glycol block copolymer. Matrices may be produced from proteins (e.g. fibrin and collagen), polymers (e.g., polyvinylpyrrolidone), or hyaluronic acid. Synthetic polymers may also be used, including bioerodible polymers (e.g., poly(lactide), poly(glycolic acid), poly(lactide-co-glycolide), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates), degradable polyurethanes, non-erodible polymers (e.g., polyacrylates, ethylene-vinyl acetate polymers and other acyl substituted cellulose acetates and derivatives thereof), non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, teflon.RTM., and nylon.

The matrix of any of the preceding aspects may be cultured with any cell of the invention, or may be administered prior to, during, or after the implantation of any cell of the invention. This administration may be by any method known to the skilled artisan (e.g., injection or surgical implantation).

In some embodiments of any of the above aspects, the cells of the invention are derived from a mammalian donor (e.g., pig or primate) of a different species than the recipient (e.g., human).

By "blood vessel formation," "blood vessel engineering," or "engineering blood vessels" is meant the dynamic process that includes one or more steps of blood vessel development and/or maturation, such as angiogenesis, vasculogenesis, formation of an immature blood vessel network, blood vessel remodeling, blood vessel stabilization, blood vessel maturation, blood vessel differentiation, or establishment of a functional blood vessel network.

Methods for measuring blood vessel formation and maturation are standard in the art and are described, for example, in Jain *et al.,* (Nat. Rev. Cancer 2:266-276, 2002). During early blood vessel formation, immature vessels resemble the vascular plexus during development, by having relatively large diameters and lacking morphological

vessel differentiation. Over time, the mesh-like pattern of immature angiogenic vessels gradually mature into functional microcirculatory units, which develop into a dense capillary network having differentiated arterioles and venules.

The number of blood vessel segments and total length of blood vessels per unit tissue area increases, accompanied by a decrease in mean vessel diameter as the blood vessels are remodeled. The total volume of blood vessels per unit tissue area typically does not change during the remodeling process. The blood vessel size distribution typically narrows with the remodeling of the vessel network.

By "angiogenesis" is meant the growth of new blood vessels originating from existing blood vessels. Methods for measuring angiogenesis are standard, and are described, for example, in Jain *et al.* (Nat. Rev. Cancer 2:266-276, 2002). Angiogenesis can be assayed by measuring the number of non-branching blood vessel segments (number of segments per unit area), the functional vascular density (total length of perfused blood vessel per unit area), the vessel diameter, or the vessel volume density (total of calculated blood vessel volume based on length and diameter of each segment per unit area).

By "vasculogenesis" is meant the development of new blood vessels originating from stem cells, angioblasts, or other precursor cells. These stem cells can be recruited from bone marrow endogenously or implanted therapeutically.

By "blood vessel maturation" is meant the structural remodeling and/or differentiation of an immature blood vessel network. In some embodiments, blood vessel maturation includes the elimination of extraneous vessels. In other embodiments, blood vessel maturation includes forming a network of blood vessels of different sizes and wall structures (e.g., capillaries, venules, veins, arterioles, and/or arteries). In some embodiments, a mature functional blood vessel network includes some vessels having at least two or more layers, including an endothelial cell layer, a basement membrane, and a perivascular cell layer. In some embodiments, a mature functional blood vessel network includes small arteries and arterioles, which decrease their size with branching, typically

40-15 μm in diameter, terminal arterioles, immediate upstream of capillaries, which typically range in size from 10-15 μm in diameter, capillaries, which typically range in size from 5-10 μm in diameter, post-capillary venules, which typically range in size from 10-20 μm, collecting venules, which are typically range in size from 15-25 μm, and venules and small veins which increase their size with gathering typically 20 – 50 μm in diameter. Optionally, the functional blood vessel network is integrated into a larger circulatory system that includes large veins and arteries.

By "microvascular scaffold" is meant a network of blood vessels. In one preferred embodiment, a microvascular scaffold is an isolated immature network of capillaries, not yet perfused, that persists for at least 24 or 48 hours. More preferably, a microvascular scaffold is a perfused network of functional blood vessels capable of supplying oxygen and nutrients to a tissue or organ and carrying away waste products. In another preferred embodiment, a microvascular scaffold is a mature network of stable differentiated blood vessels that comprises arterioles, venules, and/or other blood vessels that is integrated into the circulatory system of a tissue, organ, or mammal.

By "matrix" is meant the substance that fills the spaces between isolated cells in culture. For some applications, a matrix is an adhesive substrate used to coat a glass or plastic surface prior to cell culture. For some applications, cells are embedded in a matrix, or injected into a matrix already implanted at a desired site. For other applications, a matrix provides a physical support and an adhesive substrate for isolated cells during *in vitro* culturing and subsequent *in vivo* implantation. The matrix configuration is dependent on the tissue that is to be treated, repaired, or produced, but desirably, the matrix is a pliable, biocompatible, porous template that allows for vascular growth.

By "organ" is meant a collection of cells that perform a biological function. In one embodiment, an organ includes, but is not limited to, bladder, brain, nervous tissue, glial tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid,

trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and cartilage. The biological function of an organ can be assayed using standard methods known to the skilled artisan.

By "parenchymal cell" is meant a cell that constitutes the essential part of an organ as distinguished from associated connective tissue, blood vessels, and supporting cells.

By "perfused" is meant filled with flowing blood.

By "tissue" is meant a collection of cells having a similar morphology and function.

By "deficiency of a particular cell-type" is meant fewer of a specific set of cells than are normally present in a tissue or organ not having a deficiency. For example, a deficiency is a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% deficit in the number of cells of a particular cell-type (e.g., parenchymal cells, preadipocytes, adipocytes not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, endothelial cells, endothelial precursor cells, or fibroblasts) relative to the number of cells present in a naturally-occurring, corresponding tissue or organ. Methods for assaying cell-number are standard in the art, and are described in (Bonifacino et al., Current Protocols in Cell Biology, Loose-leaf, Jossey-Bass a Wiley Co., San Francisco, CA, 1999; Robinson et al., Current Protocols in Cytometry Loose-leaf, Jossey-Bass a Wiley Co., San Francisco, CA, October 1997).

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation).

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a polynucleotide molecule encoding (as used herein) a polypeptide of the invention.

By "positioned for expression" is meant that the polynucleotide of the invention (e.g., a DNA molecule) is positioned adjacent to a DNA sequence which directs

transcription and, for proteins, translation of the sequence (i.e., facilitates the production of, for example, a recombinant polypeptide of the invention, or an RNA molecule).

By "immunological assay" is meant an assay that relies on an immunological reaction, for example, antibody binding to an antigen. Examples of immunological assays include ELISAs, Western blots, immunoprecipitations, and other assays known to the skilled artisan. Immunological assays can be used in standard methods to reassure the function of a tissue or organ. Immunological assays can be used in standard methods to measure the function of a tissue or organ.

The invention provides methods and compositions for repairing diseased or damaged organs, either *in vivo*, or *ex vivo*, provides for improved methods of transplantation by enhancing the integration of the transplanted tissue or organ into the host's circulatory system, and provides for improved engineered tissues and organs containing functional microvascular scaffolds. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Brief Description of the Drawings

The application file contains drawings executed in color (Figures 11 and 12). Copies of this patent or patent application with color drawings will be provided by the Office upon request and payment of the necessary fee.

Figures 1A-1J illustrate angiogenesis and vessel remodeling during adipogenesis. Figure 1A is a picture of a mouse dorsal skin chamber following 3T3-F442A cell implantation. Figure 1B is a low power microscopic image of Figure 1A. Figures 1C-1F are high power microscopic images of fluorescence contrast enhanced blood vessels at 7 days (Figure 1C), 14 days (Figure 1D), 21 days (Figure 1E), and 28 days (Figure 1F) after preadipocyte implantation. Figures 1G-1J are graphs of the quantitative analysis of blood vessels during adipogenesis: number of vessel segments in the high power view field (Figure 1G); vascular length density (Figure 1H); vessel diameter (Figure 1I); and calculated blood vessel volume (Figure 1J) ($n = 7$).

Figures 2A-2H illustrate angiogenesis induced by 3T3-F442A preadipocyte cells (Figures 2A and 2B) and NIH 3T3 fibroblast cells (Figures 2C and 2D). Figures 2A-2D are pictures of a mouse dorsal skin chamber following cell implantation. Vessels were visualized at day 14 after implantation by fluorescence microscopy using FITC dextran (2M Dalton). Figures 2E-2H are graphs of quantitative analysis of blood vessels: number of vessel segments in the view field (Figure 2E); vascular length density (Figure 2F); vessel diameter (Figure 2G); and calculated blood vessel volume (Figure 2H). Closed diamonds denote 3T3-F442A preadipocytes ($n = 7$); closed squares denote NIH 3T3 fibroblasts ($n = 4$).

Figures 3A-3D are vessel diameter histograms during adipogenesis. Each segment in Figure 3 was categorized to a group depending on its diameter and shown as cumulative frequency distribution. Figure 3A is a vessel diameter histogram at day 7. In this histogram, segment diameters were distributed over a wide range. Figure 3B and Figure 3C are vessel diameter histograms at days 14 and 21, respectively. The distribution of vessel diameter shifted leftward and the range became narrower as a result of vessel remodeling with continued adipogenesis. Figure 3D is a vessel diameter histogram at day 28. In this histogram, most segments (92%) were distributed from 3 to 9 μm in diameter.

Figures 4A-4C are pictures of preadipocyte differentiation. Figure 4A is a transillumination image of differentiated adipocytes *in vitro*. Figure 4B is a fluorescence image of differentiated adipocytes *in vivo*. Figure 4C is a picture showing the effect of PPARγ inhibition on adipogenesis. Preadipocytes (1×10^5 cells) were plated in a 6-well plate and transfected at a multiplicity of infection of 10^4 plaque forming units / cell using mock- (upper panels) or PPARγ-dominant negative adenovirus (lower panels) and differentiation was promoted by using media containing 10% FBS. Oil Red O (0.3%, 1 hour at room temperature, Sigma., St. Louis, MO) staining was performed at day 12 after transfection (magnification, × 20), confirming the inhibition of adipogenesis by the PPARγ-dominant negative construct in murine preadipocytes.

Figure 5 is a table of angiogenic gene array analysis performed using Mouse Angiogenesis GEArray Q Series (Superarray Inc., Bethesda, MD), which contains 96 genes known to be involved in angiogenesis, according to the manufacturer's instructions. "(+)" denotes detectable; in this case, intensity was higher than background, and hybridization was confirmed by visual inspection. "N.D." denotes not detectable; in this case, intensity was lower or close to background. Samples are from cultured 3T3-442A cells. Preadipocytes were cultured in maintenance media (10% FCS). "PPAR-DN" denotes PPARγ dominant negative mutant receptor transduced cells cultured in maintenance media. Adipocytes were cultured in differentiation media (10% FBS). More than 2-fold difference in normalized intensity compared to preadipocytes is noted.

Figures 6A-6H illustrate the effect of PPARγ inhibition on angiogenesis. Figures 6A-6D are fluorescence images of blood vessels at 21 days (Figures 6A and 6C), and 28 days (Figures 6 Band 6D) after mock- (Figures 6A and 6B) and PPARγ dominant negative- (Figures 6C and 6D) transfected preadipocytes implantation. Figures 6E-6H are graphs of the quantitative analysis of blood vessels: number of vessel segments in the high power view field (Figure 6E); vascular length density (Figure 6F); vessel diameter (Figure 6G); and calculated blood vessel volume (Figure 6H). There was no difference between two different control cells, mock-transfected preadipocytes ($n = 3$) and EF1a-GFP 3T3-F442A cells ($n = 3$). Thus, these two groups are combined as control for data presentation and statistical analysis. Filled circle, control ($n = 6$); open square, PPARγ dominant negative–transfected preadipocytes ($n = 5$). * $P < 0.01$ as compared with corresponding control by two-tailed t-test.

Figure 7 is a picture of VEGF mRNA expression during adipocyte differentiation. Differentiation was initiated when the cell became confluent by addition of differentiation media. For control, the cells were cultured in the maintenance media without insulin. At day 8 and 12 after replacement of the media, total cellular RNA was obtained from 3T3-F442A adipocytes, and 10 μg aliquots were electrophoresed, blotted, and hybridized to the VEGF cDNA, 18S and 28S as described herein.

Figure 8 is a table of PCR primers (SEQ ID NOs: 1-25). Northern blots were probed with PCR-generated cDNA fragments. Nested primers were used to generate specific amplification products. Primers for PCR were synthesized based on Ang1, Ang2, and aP2 mouse sequences (GenBank accession numbers AAB50558, NM_007426 and NM_024406). Primers for RT-PCR were synthesized based on the GenBank sequence information. These primers were designed to amplify fragments of about 300 basepairs. Twenty five cycles each of 20 seconds at 93°C, 20 seconds at 55°C, and 30 seconds at 72°C were performed. PCR products were resolved by electrophoresis on a 2% agarose gel. The gel was stained with ethidium bromide, and bands were visualized on an UV transilluminator.

Figures 9A and 9B illustrate the effect of VEGF on preadipocyte differentiation and proliferation. To investigate the effects of VEGF on the in vitro differentiation of preadipocytes, 3T3-F442A cells were grown to confluence in media supplemented with calf serum (FCS, maintenance media), and exposed to increasing concentrations of murine recombinant $VEGF_{164}$ (R&D Systems, Minneapolis, MN) from 0 - 100 ng/ml. Mouse recombinant $VEGF_{164}$ did not induce differentiation in preadipocytes cultured in 10% FCS (maintenance media), and did not increase the differentiation rate in cells treated with 10% FBS (differentiation media) (Figure 9A).

For proliferation assays, 500 preadipocytes and fibroblasts were plated in 96 well plates, and mouse recombinant $VEGF_{164}$ (50 ng/ml) and PBS were added (Figure 9B). An MTT assay was performed at day 4, when the cells were still subconfluent in all wells. Culture media were changed with 100 μl of fresh media, and 10 μl of sterile tetrazolium salt, MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, Sigma) was added in each well and incubated for four hours at 37°C. Finally, 100 μl of 10% SDS were added, and after incubation at 37°C overnight, the plate was read at 490 nm. The optical density values were normalized to that of the PBS treated cells and used as a measure of viability.

Figure 10 is a schematic illustration of proposed mechanisms of reciprocal regulation of adipogenesis and angiogenesis.

Figure 11 is a photomicrograph showing engineered microvessel growth detected by multiphoton laser scanning microscopy 37 days after implantation of human umbilical vein endothelial cells HUVEC and 3T3-F442a co-cultured cells. HUVEC are shown in green (EGFP labeled), and functional blood vessels, visualized by injected rhodamine dextran, are shown in red.

Figure 12 is a picture showing processes of tissue engineered microvessel growth detected by multiphoton laser scanning microscopy 35 day after implantation of co-cultured HUVEC and 10T1/2 cells. HUVEC are shown in green (EGFP labeled), and functional blood vessels, visualized by injected rhodamine dextran, are shown in red.

Description of the Invention

The present invention features improved methods and compositions for tissue and organ transplantation, the repair of diseased or damaged tissues and organs, and replacement tissue and organ engineering.

As reported in more detail below, human umbilical vein endothelial cells embedded in three-dimensional matrices formed functional vessels *in vivo*, and the engineered vessels were stabilized by co-implantation with mesenchymal precursor cells (10T1/2) or by preadipocytes (3T3-F442A). In addition, preadipocytes or differentiated adipocytes enhanced and recruited host vessels to infiltrate the three-dimensional matrices. The discovery that preadipocytes induced vigorous angiogenesis *in vivo*, and that the newly formed vessels subsequently remodeled into a mature network consisting of arterioles, capillaries, and venules provides for improved therapeutic methods of tissue and organ transplantation, methods for stabilizing and repairing damaged tissues or organs, and improved methods for the engineering of tissues and organs.

For successful tissue and organ transplantation, the grafted tissue or organ must be rapidly integrated into the host's circulatory system. Applicants' discovery that

preadipocytes and mesenchymal precursor cells induced not only angiogenesis but also subsequent vessel remodeling and maturation provides for improved methods of tissue and organ transplantation. Administering preadipocytes or mesenchymal precursor cells to grafted tissue or organs induces blood vessel formation and a functional blood vessel network, and enhances integration of the graft into the recipient's circulatory system.

Many patients remain on transplantation waiting lists for months or even years. Thus, a need exists for therapeutic methods that would stabilize a patient's damaged organ while the patient waits for transplantation or repair a diseased or damaged organ. The discovery that administering preadipocytes or mesenchymal cells induces blood vessel formation and a functional blood vessel network provides for the stabilization or repair of damaged or diseased tissues and organs, thereby improving tissue or organ function. Such methods may prolong organ life while a patient waits for a donor organ, or may obviate the need for transplantation.

In addition, shortages in organ donation could be offset if transplantable tissues or organs could be engineered. Successful tissue and organ engineering has been limited by failures in engineering functional blood vessels. The discovery that endothelials cells formed functional microvascular scaffolds when cultured with preadipocytes or mesenchymal precursor cells provides for the culture of microvascular scaffolds whose interstices are filled with parenchymal cells from virtually any target-tissue thus allowing the engineering of replacement tissues and organs.

These methods are described further below.

Mouse Model of Angiogenesis

Methods for producing an engineered microvascular scaffold were developed using a mouse model of angiogenesis. This model was developed using an established preadipocyte cell line (3T3-F442A) that is able to differentiate into mature adipocytes in culture (Gregoire *et al.*, Physiol. Rev. 78:783-809, 1998). 3T3-F442A cells give rise to vascularized fat pads in immunodeficient mice (Mandrup *et al.*, Proc. Natl. Acad. Sci.

U.S.A. 94:4300-4305, 1997). 3T3-F442A preadipocytes (a generous gift from Dr. Bruce Spiegelman, Dana-Farber Cancer Institute, Boston, MA) and their parental cell line (NIH 3T3 fibroblasts) were maintained in Dulbecco's Minimum Essential Medium (DMEM, Gibco BRL, Grand Islands, NY), supplemented with 10% calf serum, glucose, L-glutamine, penicillin, and streptomycin. For cell identification *in vivo*, preadipocytes were transfected by the calcium phosphate method with *GFP* under the EF1a promoter; these cells are referred to as GFP/3T3-F442A.

These preadipocytes were implanted in the dorsal skin-fold chamber (Jain *et al.*, Nat. Rev. Cancer 2:266-276, 2002) of male, 8-12-weeks-old severe combined immunodeficient (SCID) mice to monitor angiogenesis during fat formation. In particular, dense cell pellets containing 2 x 10^5 mouse preadipocytes (or NIH 3T3 fibroblasts as a control) were implanted in the center of the dorsal skinfold chamber. These mice were bred and maintained in a defined flora facility. *In vivo* microscopy was performed 1-2 times a week for as many as four weeks after the implantation. Implants were then analysed for vascular parameters as described previously (Jain *et al.*, *supra*).

For each animal, the implant was analysed at five randomly chosen locations per time point. The number of non-branching blood vessel segments (number of segments per unit area), the functional vascular density (total length of perfused blood vessel per unit area), the vessel diameter, and the vessel volume density (total of calculated blood vessel volume based on length and diameter of each segment per unit area) were determined as described elsewhere (Jain *et al.*, *supra*). Angiogenesis and subsequent vessel remodeling were analyzed following the implantation of NIH 3T3, 3T3-F442A, GFP/3T3-F442A, or GFP/3T3-F442A cells infected with a recombinant adenovirus encoding a PPARγ dominant negative mutant receptor or mock adenovirus (Gurnell *et al.*, J. Biol. Chem. 275:5754-5759, 2000).

Characterization of Angiogenesis in Mouse

Mice with transplanted preadipocytes displayed reddened tissue at sites of active angiogenesis (Figure 1A). Angiogenic vessels were detected in implanted 3T3-F442 cell pellets located on top of host subcutaneous tissues and striated muscle which contained pre-existing host vessels (Figure 1B). The angiogenic vessels were specifically induced by the 3T3-F442A preadipocytes since control fibroblasts (NIH 3T3 cells) failed to induce detectable vessel formation (Figures 2A-2H).

New vessels induced by the preadipocyte implant appeared immature, resembling the vascular plexus during development, having relatively large diameters and lacking morphological vessel differentiation (Figure 1C). Over time, the vessel network induced by the preadipocyte implant gradually matured (Figures 1C-1F). Mesh-like patterns of angiogenic vessels developed into a dense capillary network (Figure 1C-1E), with differentiated arterioles and venules (Figure 1F). The number of blood vessel segments (Figure 1G) and total length of blood vessels per unit tissue area (Figure 1H) increased, accompanied by a decrease in mean vessel diameter (Figure 1I) as the blood vessels remodeled. The total volume of blood vessels per unit tissue area did not change during the remodeling process (Figure 1J). The blood vessel size distribution narrowed with the remodeling of the vessel network (Figures 3A-3D). These findings indicate that preadipocytes induce angiogenesis *in vivo* and that these vessels are remodelled into an efficient network with mature vessel architecture characterized by small diffusion distance from vessels to parenchymal cells..

To clearly distinguish between implanted cells and host-derived cells *in vivo*, 3T3-F442A cells were detected by constitutively expressing the green fluorescent protein (GFP) gene under the control of the *EF1*α promoter. Cytoplasmic GFP fluorescence allowed the detection of the implanted cells *in vivo*. The implanted preadipocytes began to differentiate into adipocytes several days after implantation and most of the cells acquired a mature phenotype after four weeks. Differentiation into adipocytes was accompanied by the accumulation of triglyceride-containing vesicles in the cell cytosol

(Figure 4A), which exhibited a granular fluorescence (Figure 4B). Most of the cells acquired a mature phenotype after four weeks, and adipocyte differentiation was confirmed by the expression of the adipocyte-specific genes *aP2* and *CD36* (Figure 5), (Bernlohr *et al.*, Biochem. Biophys. Res. Commun. 132:850-855, 1985; Spiegelman *et al.*, J. Biol. Chem. 258:10083-10089, 1983; and Abumrad *et al.*, J. Biol. Chem. 268:17665-17668, 1993).

Requirement of PPARγ for Adipogenesis

Activation of PPARγ is required for adipocyte differentiation (Lazar, Genes & Dev. 16:1-5, 2002 and Willson *et al.*, Annu. Rev. Biochem. 70:341-367, 2001). To characterize the link between preadipocyte differentiation and angiogenesis, a dominant negative peroxisome proliferator-activated receptor γ (PPARγ) mutant construct was introduced into 3T3-F442A cells prior to implantation using an adenoviral vector, as described previously (Gurnell *et al.*, J. Biol. Chem. 275:5754-5759, 2000). Expression of the mutant receptor prevented the differentiation of 3T3-F442A cells *in vitro* (Figure 4B). Control mock-transfected preadipocytes formed fat tissue and induced extensive angiogenesis when implanted *in vivo* (Figures 6A, 6B, and 2E-2H), while cells expressing the PPARγ dominant negative mutant receptor formed no fat tissue and induced reduced angiogenesis when implanted *in vivo*. Underlying host blood vessels remained visible for the duration of the experiment (Figures 6C and 6D) when PPARγ dominant negative cells were implanted. These dominant negative cells remained undifferentiated and expressed lower messenger RNA levels of *aP2*. Thus, the activation of PPARγ is required for adipogenesis and subsequent angiogenesis *in vivo*.

Expression of VEGF and other angiogenesis related factors in adipose tissue

Angiogenesis often precedes adipose tissue formation in developing tissue. VEGF is the most potent and critical angiogenic factor in both physiological and pathological angiogenesis. VEGF is highly expressed in adipose tissue and its expression increases during PPARγ ligand and other stimuli-induced differentiation of preadipocytes into adipocytes (Figures 7 and 5) (Zhang *et al.*, J. Surg. Res. 67:147-154, 1997; Claffey *et al.*, J. Biol. Chem. 267:16317-16322, 1992; Soukas *et al.*, J. Biol. Chem. 276:34167-34174, 2001; and Emoto *et al.*, Diabetes 50:1166-1170; 2001). In agreement with these data, expression of *VEGF* and various other angiogenesis-related genes was found (Figure 5) in 3T3-F442A cell-derived tissue *in vivo*.

To analyze expression of angiogenesis-related genes *in vivo*, 1.5 x 10^7 cells suspended in 100 μl of PBS were injected into the flank of SCID mice. For the anti-adipogenesis studies, mice were divided into three groups with the following cell implants: GFP/3T3-F442A, GFP/3T3-F442A expressing PPARγ dominant negative, and GFP/3T3-F442A mock-transfected. For the anti-angiogenesis experiments, GFP/3T3-F442A cells were implanted in three groups of mice. Fat pad formation was allowed to occur for four weeks, then mice were sacrificed, and the tissue was harvested. The tissue formed by the implanted preadipocytes was recovered using microscissors and fluorescence microscope-guided dissection. Tissue samples were snap-frozen for subsequent RNA extraction.

Total RNA was extracted from the cells and the recovered tissue samples using Triazol (Gibco BRL, Grand Islands, NY), following the protocol recommended by the manufacturer. The GFP expression in tissue samples was confirmed by RT-PCR. Primers for RT-PCR and Northern probes are shown in Figure 8. Ten micrograms of total RNA was separated on a1% agarose / 1x MOPS / 2% fomaldehyde gel, transferred to nylon membranes in 10 x SSC, and UV cross-linked to the membrane. Northern blots were hybridized with random-primed ^{32}P-labeled probes in QuickHybr Solution (Stratagene, La JollaCA) at 68°C for one hour. Hybridized blots were washed twice at high stringency in a solution of 0.1 x SSC / 0.1% sodium dodecylsulfate (SDS) at 55°C. Autoradiography was performed for 1-2 days using a Kodak X-Omat AR film.

Effect of exogenous VEGF on adipogenesis *in vitro*

Adipose cell differentiation and proliferation *in vitro* were not significantly affected by exogenous VEGF, even at doses as high as 100 ng/ml $rmVEGF_{165}$. The effect of VEGF on adipose cells in culture is shown in Figures 9A and 9B. Thus, VEGF signaling may not directly mediate adipogenesis; although, for example, neuropilin-1 was detected in preadipocytes (Figure 5). Thus, other molecular and microenvironmental

changes associated with angiogenesis and/or secondary to VEGF signaling may potentiate adipogenesis *in vivo*.

Interplay between adipose tissue formation, angiogenesis, and vessel remodeling

These data illustrate the complex interplay between adipose tissue formation, angiogenesis, and vessel remodeling. Angiogenesis was needed for efficient preadipocyte differentiation, but angiogenesis was not triggered without PPARγ activation and subsequent adipocyte differentiation. To analyze the cyclic feedback mechanisms gene array analysis on these tissues was performed at various time points in preadipocytes, adipocytes, and PPARγ dominant negative expressing cells. (Figure 5). *Ang-1* expression was increased in 3T3-L1 cells, a preadipocyte cell line, during adipogenesis (Stacker *et al.*, Growth Factors 18:177-191, 2000), but *Ang-1* was not detectable in mature adipose tissue (Figure 5). *Ang-2* was expressed in both preadipocytes and adipocytes *in vitro* and was significantly upregulated in PPARγ-dominant negative expressing cells (Figure 5). Adipogenesis may be mediated by (i) the auto- and paracrine effects of other angiogenic growth factors on preadipocytes or (ii) the interactions between the matrix associated with angiogenic vessels and preadipocytes (Figure 5; Varzaneh *et al.*, Metabolism 43:906-912, 1994 and Lilla *et al.*, Am. J. Pathol. 160:1551-1554, 2002).

A salient observation emerging from this study comes from the remodeling and maturation of angiogenic vessels. While aberrant angiogenesis occurs during tumor angiogenesis, driven by excess and/or unbalanced angiogenic factors (Carmeliet *et al.*, Nature 407:249-257, 2000), in this model system new blood vessels mature into a normal network during adipose tissue formation. This is remarkable given that "normal" vasculature is rarely generated in currently available tissue engineering models. Furthermore, these results suggest that the molecular and metabolic microenvironment associated with functional, mature blood vessels potentiates preadipocyte differentiation and adipose tissue formation (Figure.10). Thus, it is likely that the generation of normal

microcirculatory units is indispensable for organogenesis. The new adipogenesis-organogenesis model described herein is ideal to address the mechanisms of normalization and maturation of blood vessels, and to develop and test novel strategies for tissue engineering, organogenesis, and therapeutic blood vessel formation and blood vessel engineering.

Angiogenesis in Three-Dimensional Matrices

The ability of preadipocytes to stabilize engineered vessels was analyzed *in vivo*. Three-dimensional matrices containing co-cultures of implanted human umbilical vein endothelial cells (HUVEC), transfected with EGFP and either 3T3-F442a, preadipocytes, or mesenchymal precursor cells was compared to HUVEC-only implants in a mouse cranial window preparation and in a dorsal skinfold chamber.

Craniotomy and Cranial Window (CW) Preparation

Cranial windows were implanted into mice as previously described (Yuan *et al.*, Cancer Research 54: 4564-4568, 1994). SCID mice (25-30 g) were anesthetized; the head of the mouse was fixed by a stereotactic apparatus, and a longitudinal incision was made between the occiput and forehead. The skin was cut in a circular manner on top of the skull, and the periosteum underneath was scraped off to the temporal crests. A 6-mm circle was drawn over the frontal and parietal regions of the skull bilaterally. Using a high speed air-turbine drill (CH4201S; Champion Dental Products, Placentia, CA) with a burr-tip, 0.5 mm in diameter. A groove was made on the margin of the drawn circle, which was then made thinner until the bone flap loosened. Cold saline was applied during the drilling process to avoid thermal injury of the cortical regions. Using a malis dissector, the bone flap was then separated from the dura mater underneath. After removal of bone flap, the gelfoam was placed on the cutting edge and the dura matter was continuosly kept moist with physiological saline. A nick will be made close to the sagital sinus. Iris microscissors were passed through the nick. The dura and arachnoid

membranes were cut completely from the surface of both hemispheres, and an 8 mm cover glass was glued to the bone with histocompatible cyanoacrylate glue. The mouse was then allowed to recover.

Between seven and ten days after surgery, the cover glass was removed and a piece of gel 3 mm in diameter was put in the center of the window, which was then re-sealed as previously described. Angiogenesis and maturation processes were monitored.

Dorsal Skinfold Chamber (DSC) Preparation

The dorsal skinfold chamber preparation was performed as previously described (Leunig *et al.*, Cancer Research 52: 6553-6560, 1992). SCID mice (25-30 g) were anesthetized. Two symmetrical titanium frames (weight 3.2 g each), which were mirror images of each other (Workshop, Department of Radiation Oncoclogy, MGH) were implanted such that a layer of skin was sandwiched between them. The outer layer of the skin was removed in a 15 mm diameter circle to expose the epidermis, subcutaneous tissue, and striated muscle, which was then covered with a glass coverslip secured into one of the frames by a snap ring. The mouse was allowed to recover from surgery for one day. On the second day after surgery, the mouse was placed in a polycarbonate tube 25 mm in diameter. The snap ring and glass coverslip were removed, and a disk of gel (1mm in height and 3 mm in diameter) was placed in the chamber. A coverslip was then set into place and secured with a snapring. Angiogenesis and maturation processes were then monitored.

Three-dimensional Cell Constructs

In vitro derived three-dimensional cell constructs of endothelial cells and preadipocytes cultured in a collagen gel were transferred into cranial windows and dorsal skinfold chambers as described above. The three-dimensional cell constructs were produced as follows.

The three dimensional matrix was produced according to the manufacturer's protocol. Type 1 collagen (1.5 mg/ml) was mixed with human plasma fibronectin (90 μg/ml) in 25 mM Hepes and special growth medium for endothelial cells (EGM) at 4°C, and the pH was adjusted to 7.5 using NaOH. Cultured EGFP transduced HUVEC and/or 3T3-F442a (or Swiss 3T3) were trypsinized, and the cells were counted. For gels containing co-cultures of HUVEC and 3T3-F442a (or Swiss 3T3) cells, 0.8 x 10^6/ml and 0.2 x 10^6/ml collagen solutions, respectively, were used per 1 ml of gel. For control gels ontaining only HUVEC, 1 million HUVEC were used for 1 ml of gel. The cultured cells were placed into a 15 ml culture tube and spun in a centrifuge to pellet the cells. Then, the supernatant was removed. The cell pellet was then resuspended in 1 ml gel solution, placed in the well of a 12 well plate, and incubated at 37 °C and 5% CO_2 for thirty minutes to allow the gel to polymerize. One ml of endothelial cell growth media modified MCDB131 (EGM) (Cambrex Bio Science Walkerville Inc.Walkersville, MD, CC-3024) media was then added to the well. The cells in the gel were then cultured for twenty-four hours *in vitro*. The disc-like pieces of gel (3 mm in diameter and 1 mm in height) were transferred to the observation sites (cranial window or dorsal skin fold) approximately twenty-four hours after formation. The cultures containing EGFP transduced HUVEC and 10T1/2 mesenchymal precursor cells were prepared in the same way.

Anastamosis and Microvascular Function

Using intravital and two-photon microscopy, (i) anastomosis between the artificial vessels and the host microvascular system and (ii) microvascular function of the perfused artificial constructs were assessed. Following anaesthesia, intravital microscopy was performed as previously described (Leunig *et al.*, Cancer Research 52: 6553-6560, 1992). Cranial window-bearing mice were fixed on an observation stage to hold the window in position during measurements. Dorsal skinfold mice were positioned in a polycarbonate tube approximately 24 mm in diameter on a polycarbonate stage. The stage was then

placed under a microscope. Daily observations were performed using either a 1.25x objective (NA 0.035, Plan Neofluor; Zeiss, Oberkochen, Germany) and a microscope (Axioplan, Zeiss) with a 37-fold magnification on the screen or a 20x long working distance objective (NA 0.4; LD Achroplan) with a final magnification of 570-fold. For general morphology, a transillumination technique (12 V, 100 W halogen lamp, Zeiss), using a green filter for enhancing black/white photomicrography and a conversion filter for converting artificial light of 3200 K into daylight of 5500 K, was used. GFP-expressing endothelial cells were visualized using epifluorescence and fluorescence filters. Observations (intensified CCD camera, C2400-88; Hamamatsu Photonics K.K., Hamamatsu, Japan) were recorded on a videocassette recorder (AG-6500; Panasonic, Secaucus, NJ) at a rate of 60 frames per second.

Multiphoton laser-scanning microscopy

To determine functionality of engineered vessels, the tail vein was injected with tetramethylrhodamine dextran. Images were taken with multiphoton laser-scanning microscopy (MPLSM). This technology allowed for three-dimensional, high-resolution imaging of endothelial cells at depths extending 400 μm below the surfance of the window. MPLSM was described previously (Brown *et al.*, Nature Medicine 7: 864-868, 2001; Padera *et al.*, Molecular Imaging 1: 9-15, 2002). Briefly, MPLSM consists of a MILLENIAX PUMPED TSUNAMI TI:sapphire laser (Spectra-Physics, Mountain View, California). All data were obtained using a 810–850 nm light. Power at the sample was estimated to be 1–5 mW. The scanhead was a MRC600 with IR optics (Bio-Rad, Hemel Hempstead, England) on a Zeiss AXIOSKOP20 microscope (Zeiss, Jena, Germany) with a LOMO 30x/0.9NA/water (Vermont Optechs, Charlotte, Vermont) and Zeiss 20x/0.5NA/water and 20x/0.4NA/air objective lenses. Non-descanned detection was performed with HC125-02 multiplier (PMT) (Hamamatsu Photonics, Bridgewater, New Jersey). Image analysis was performed on SCIONIMAGE software (Scion, Frederick,

Maryland). After images were taken, the animals were euthanized, and the gel and surrounding tissue were removed for immunohistochemical analysis.

Angiogenesis in Three Dimensional Matrices with Preadipocytes and HUVEC

Initially, HUVEC cells co-cultured in collagen matrix with preadipocyte 3T3-F442a displayed a small spindle-shaped morphology. When implanted into an SCID mouse cranial window, HUVEC formed a capillary-like network. At ten days after implantation, blood flow was observed in a small number of engineered vessels (Figure 11). The number of perfused vessels increased over the course of the experiment. In addition, host vessels were recruited into the implants. These results show that endothelial cells (HUVEC) embedded in three-dimensional matrices are able to form functional vessels *in vivo* and the engineered vessels are stabilized by co-implantation with adipocyte precursor cells 3T3-F442a.

Angiogenesis in Three-Dimensional Matrices with Mesenchymal Precursor Cells and HUVEC

As in the preadipocyte matrix, initially HUVEC co-cultured with 10T1/2 mesenchymal precursor cells had a small spindle-shaped morphology. After implantation, HUVEC formed a capillary-like network. At ten days after implantation, blood flow was observed in a small number of engineered vessels (Figure 12). The number / proportion of perfused vessels was increased thereafter in the HUVEC-10T1/2 co-implant group. On the other hand, HUVEC alone implants had fewer perfused vessels and capillary-like structures, and these vessels had virtually disappeared at 7 weeks post-implantation. These results showed that endothelial cells (HUVEC) embedded in three-dimensional matrices were able to form functional vessels *in vivo* and that the engineered vessels were stabilized by co-implantation with mesenchymal precursor cells (10T1/2).

Methods for Constructing Engineered Microvascular Scaffolds

Using preadipocytes and mesenchymal cell precursors to stabilize and enhance artificially generated blood vessel networks provides improved artificially engineered tissues having a functional microvascular scaffold. Blood or lymph vascular endothelial cells or endothelial precursor cells are used to prepare engineered blood and lymph vessels. Blood or lymph vascular endothelial cells form the primary layer of the engineered vessel; such cells are obtained, for example, from established endothelial cell lines, freshly prepared primary culture endothelial cells, or endothelial cells generated from stem cells *in vitro*. Sources of such embryonic stem cells include, but are not limited to, bone marrow derived stem cells, cord blood derived cells, HUVEC, lymphatic endothelial cells , embryonic stem cells , and endothelial pregenitor cells .

Cells that are useful for stabilizing the engineered vessels include perivascular cells (e.g., pericytes); vascular smooth muscle cells; mesenchymal precursor cells, (e.g., embryonic stem cells, 10T1/2 cells, preadipocytes, (e.g., TA1, 3T3-L1, 3T3-F442A, or Ob17) fibroblasts; murine embryonic fibroblasts; fibroblast cell lines (e.g., NIH 3T3, Swiss 3T3, BalbC 3T3); and tumor activated stromal cells (e.g., GFP positive cells isolated from tumors grown in VEGF-GFP mice, EF1a-GFP mice, or Tie2-GFP mice).

The interstices within the engineered microvascular networks can be filled with parenchymal cells from virtually any organ. Because many cell-types can be expanded *in vitro,* grafts can be made using a limited number of cells (e.g., 100, 500, 1000, 10,000, 100,000, 1,000,000, 10,000,000, or 100,000,000), which represent a small percentage (e.g., 0.0001%, 0.001%, 0.005%, 0.01%, 0.05%, 0.10%, 1.0%, 2.0%, or 5.0%) of the cells present in a naturally-occurring tissue or organ. Exemplary parenchymal cells for organogenesis include, hepatocytes, myocytes (e.g., cardiac or skeletal muscle myocytes), keratinocytes, osteocytes, chondrocytes, islet cells, nerve cells, astrocytes, glial cells from the central or peripheral nervous system, preadipocytes derived from fat or breast tissue, and adipocytes. Such cells might be obtained from the intended implant recipient (an autograft), from a donor (allogeneic graft), or from a cell line. One

particular advantage of autografts is that the grafted tissue does not induce an immune response because the grafted cells are recognized as self (Heath *et al.*, Trends Biotechnol, 18:17-19, 2000). In other embodiments, such cells might be obtained from a mammal of a different species (e.g., pig or primate).

Cell Isolation

One aspect of the invention pertains to a replacement organ that includes an engineered microvascular scaffold. The replacement organs may derived from the recipient's own tissue, derived from a different individual of the same species, or derived from a mammalian species that is different from the recipient (e.g., pig or primate).

Cells can be isolated from a number of sources, for example, from biopsies or autopsies using standard methods. The isolated cells are preferably autologous cells obtained by biopsy from the subject. The cells from biopsy can be expanded in culture. Cells from relatives or other donors of the same species can also be used with appropriate immunosuppression. Methods for the isolation and culture of cells are discussed in Fauza *et al.* (J. Ped. Surg. 33, 7-12, 1998)

Cells are isolated using techniques known to those skilled in the art. For example, the tissue or organ can be disaggregated mechanically and/or treated with digestive enzymes and/or chelating agents that weaken the connections between neighboring cells making it possible to disperse the tissue into a suspension of individual cells without appreciable cell breakage. Enzymatic dissociation can be accomplished by mincing the tissue and treating the minced tissue with digestive enzymes (e.g., trypsin, chymotrypsin, collagenase, elastase, hyaluronidase, DNase, pronase, and dispase). Mechanical disruption can be accomplished by scraping the surface of the organ, the use of grinders, blenders, sieves, homogenizers, pressure cells, or sonicators. For a review of tissue disruption techniques, see Freshney, (Culture of Animal Cells. A Manual of Basic Technique, 2d Ed., A. R. Liss, Inc., New York, Ch. 9, pp. 107-126, 1987).

Preferred cell types include, without limitation, adipocytes, preadipocytes, urothelial cells, mesenchymal cells, especially smooth or skeletal muscle cells, myocytes (muscle stem cells), cardiac myocytes, mesenchymal cells, mesenchymal precursor cells, fibroblasts, chondrocytes, fibromyoblasts, ectodermal cells ductile cells, and skin cells, hepotocytes, Islet cells, cells present in the intestine, parenchymal cells, other cells forming bone or cartilage (e.g., osteoblasts), and nerve cells.

Once the tissue has been reduced to a suspension of individual cells, the suspension can be fractionated into subpopulations. This may be accomplished using standard techniques (e.g., cloning and positive selection of specific cell types or negative selection, i.e., the destruction of unwanted cells). Selection techniques include separation based upon differential cell agglutination in a mixed cell population, freeze-thaw procedures, differential adherence properties of the cells in the mixed population, filtration, conventional and zonal centrifugation, unit gravity separation, countercurrent distribution, electrophoresis and fluorescence-activated cell sorting. For a review of clonal selection and cell separation techniques, see Freshney, Culture of Animal Cells. A Manual of Basic Techniques, 2d Ed., A. R. Liss, Inc., New York, Ch. 11 and 12, pp. 137-168, 1987).

Cell fractionation may be useful when the donor has a disease, such as cancer. Isolated cells can be cultured *in vitro* to increase the number of cells available for transplantation. The use of allogenic cells, and more preferably autologous cells, is preferred to prevent tissue rejection. However, if an immunological response does occur in the subject after implantation of the engineered organ, the subject may be treated with immunosuppressive agents, such as cyclosporin or FK506, to reduce the likelihood of rejection.

Isolated cells may be transfected. Useful genetic material may be, for example, genetic sequences that are capable of reducing or eliminating an immune response in the host. For example, the expression of cell surface antigens such as class I and class II histocompatibility antigens may be suppressed. This may allow the transplanted cells to

have reduced chance of rejection by the host. In addition, transfection could also be used for gene delivery. The cell-substrate construct can carry genetic information required for the long term survival of the host or the artificial organ.

Isolated cells can be normal or genetically-engineered to provide additional or normal function. Methods for genetically engineering cells with retroviral vectors or other methods known to those skilled in the art can be used. These include using expression vectors which transport and express nucleic acid molecules in the cells (see, for example, Goeddel et al., (Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif.,1990).

Vector DNA is introduced into prokaryotic or cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks.

Methods for Making Cell Suspensions

The isolated parenchymal cells are mixed with a matrix and injected directly, or cultured for a time with a suitable matrix polymer that may or may not have an existing microvascular scaffold. The polymer is dissolved in an aqueous solution, preferably a 0.1 M potassium phosphate solution, at physiological pH, to a concentration forming a polymeric hydrogel. The isolated cells, including an adipocyte, preadipocyte, mesenchymal precursor cell or a mesenchymal cell, an endothelial cell, and a parenchymal cell, are suspended in the polymer solution to a concentration of between 1 and 50 million cells/ml, most preferably between 5 and 10 million cells/ml.

Cells are cultured on or embedded in a matrix, or injected into a matrix already implanted at the desired site. Desirably, the matrix is a pliable, non-toxic, injectable porous template that allows for vascular growth. The pores should allow for vascular growth and the injection of cells without damage to the cells or to the patient. These are

generally interconnected pores in the range of between approximately 100 and 300 microns. The matrix should be shaped to maximize surface area, to allow adequate diffusion of nutrients and growth factors to the cells and to allow the growth of new blood vessels and connective tissue. A porous structure that is resistant to compression is preferred. The matrix configuration is dependent on the tissue which is to be treated, repaired, or produced.

Methods for Repairing Damaged Tissues and Organs

The invention features methods of repairing diseased or damaged tissues and organs. Cells (e.g., preadipocytes, adipocytes having or not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, and fibroblasts) are administered to a damaged or diseased tissue or organ. These cells induce blood vessel formation and a functional blood vessel network in the damaged tissue or organ thereby increasing blood supply to the organ, improving organ biological function, and increasing parenchymal cell proliferation. Optionally, endothelial cells or endothelial cell precursors may also be administered. These methods may stabilize a damaged tissue or organ in a patient on a transplantation waiting list; or the methods may repair a damaged or diseased tissue or organ, thereby obviating the need for transplantation.

Methods for Organ or Tissue Transplantation

The invention features improved methods for organ or tissue transplantation. Cells (e.g., preadipocytes, adipocytes having or not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, and fibroblasts) are administered to a tissue or organ (donor organ or engineered organ) prior to, during, or after transplantation. Optionally, endothelial cells or endothelial precursor cells may also be administered. The administration of one or more of these cell-types enhances blood vessel formation and a functional blood vessel network in the tissue or

organ, increases infiltration of the graft by host blood vessels, and improves the integration of the graft into the host's circulatory system.

Methods for Producing Engineered Tissues or Organs

The invention features methods of producing engineered replacement organs comprising a functional microvasculature. Cells (e.g., preadipocytes, mesenchymal cell precursors, blood and/or lymph vascular endothelial cells, pericytes, vascular smooth muscle cells, mesenchymal precursor cells, and tumor activated stromal cells) are preferably cultured in the presence of a matrix (e.g., a synthetic polymer based matrix, decellularized skin or other tissue source; collagen or other extracellular matrix gel) as described herein. Optionally, the interstices of the microvascular scaffold are filled with parenchymal cells, derived from virtually any organ of interest, to generate an engineered tissue or organ. Parenchymal cells may be introduced prior to, during, or after formation of the microvascular scaffold. Methods for producing an engineered tissue or organ may be carried out either *in vitro* or *in vivo*.

Engineered Tissues and Organs

Organs that can be produced using the methods of the invention include, but are not limited to, the bladder, brain, nervous tissue, glial tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and cartilage. Such organs are generated, for example, by culturing a pre-adipocyte, adipocyte, mesenchymal precursor, or mesenchymal cell and an endothelial cell to produce a functional microvascular scaffold.

Parenchymal cells are introduced to the culture, and allowed to fill the interstitial spaces within the microvasculature. Alternatively, parenchymal cells are introduced to the matrix before, during, or after formation of the microvascular scaffold. Most preferably, an engineered organ comprises (i) an adipocyte, preadipocyte, or a

mesenchymal precursor cell, (ii) an endothelial cell, and a (iii) parenchymal cell embedded in a matrix or cultured on the surface of a matrix. Optionally, an engineered organ may comprise only parenchymal cells and an adipocyte, preadipocyte, or a mesenchymal precursor cell. Engineered organs are useful for the treatment of a variety of diseases or disorders.

In particular, an engineered organ comprising insulin-producing cells, adipocytes, preadipocytes, mesenchymal cells, or mesenchymal precursor cells, and endothelial cells, is administered to a patient for the treatment or prevention of diabetes; oligodendroglial precursor cells, adipocytes, preadipocytes, mesenchymal cells, or mesenchymal precursor cells, and endothelial cells are administered for the treatment or prevention of multiple sclerosis. For the treatment or prevention of endocrine conditions, engineered organs that produce a hormone, such as a growth factor, thyroid hormone, thyroid-stimulating hormone, parathyroid hormone, steroid, serotonin, epinephrine, or norepinephrine may be administered to a mammal. Additionally, an engineered organ comprising epithelial cells, adipocytes, preadipocytes, or mesenchymal precursor cells, and endothelial cells are administered to repair damage to the skin, or to the lining of a body cavity or organ, such as a lung, gut, or urogenital tract. A replacement liver is generated by culturing a hepatocyte and an adipocyte, preadipocyte, or mesenchymal precursor cell, and an endothelial cell. It is also contemplated that parenchymal cells having a microvascular scaffold are administered to a mammal to treat damage or deficiency of cells in an organ, muscle, or other body structure, or to form an organ, muscle, or other body structure. Desirable organs include the bladder, brain, nervous tissue, glial tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and cartilage. Optionally, a replacement organ comprises only parenchymal cells and an adipocyte, preadipocyte, or a mesenchymal precursor cell.

Parenchymal cells are also combined with a matrix and a microvascular scaffold to form a tissue or organ *in vitro* or *in vivo* that may be used to repair or replace a tissue or organ in a recipient mammal. For example, parenchymal cells are cultured *in vitro* or *in vivo* in the presence of a matrix and a microvascular scaffold to produce a tissue or organ that is transplanted into a mammal.

Engineered Bone or Cartilage

Exemplary transplantation methods of the present invention also include repairing or replacing bone or cartilaginous tissue. Traditional bone or cartilage tissue engineering methods can be improved by administering preadipocytes, adipocytes not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, and fibroblasts to the damaged or diseased bone or cartilage *in vivo* or to a bone or cartilage transplant tissue before, during, or after the transplant tissue is administered to a mammal. Preferably, endothelial cells or endothelial precursor cells are also administered to enhance angiogensis. Traditional bone and cartilaginous tissue reconstruction methods are described, for example, in U.S. patent Nos. 6,197,061; 6,197,586; 6,228,117; 6,419,702; and 6,451,060. Engineered bone is useful for the treatment of a variety of diseases or disorders, including arthritis, cancer, congenital defects of bone or cartilage, and trauma.

It is known that connective-tissue cells, including fibroblasts, cartilage cells, and bone cells, can undergo radical changes of character. Thus, as explained by Alberts *et al.*, (Molecular Biology of the Cell 2nd Ed., pp. 987-988, 1989), a preparation of bone matrix may be implanted in the dermal layer of the skin and some of the cells there are converted into cartilage cells and others into bone cells.

A great variety of materials are useful as matrices for this purpose. For example, materials such as collagen gels, poly(D,L-lactide-co-glycolide (PLGA) fiber matrices, polyglactin fibers, calcium alginate gels, polyglycolic acid (PGA) meshes, and other

polyesters such as poly-(L-lactic acid) (PLLA) and polyanhydrides are among those suggested.

Methods for treating connective tissue disorders using engineered cartilaginous or connective tissues are described, for example, in U.S. Patent Nos.: 5,226,914; 5,041,138; 5,368,858; 5,632,745; 6,451,060; 6,197,586; and 6,197,061. In some embodiments, chondrocytes are cultured with (i) a preadipocyte, an adipocyte, a mesenchymal precursor cell, or a mesenchymal cell, and (ii) an endothelial cell or an endothelial precursor cell and then implanted. Surgical procedures related to bone tissue deficiencies vary from joint replacement or bone grafting to maxillo-facial reconstructive surgery. Such methods are known to the skilled artisan.

Engineered Soft Tissue

Traditional methods of soft tissue reconstruction, as described in U.S. Patent No. 5,716,404, can be improved by administering preadipocytes, adipocytes not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, and fibroblasts to the soft tissue to be transplanted. For example, engineered soft tissue is useful for cosmetic surgery or for reconstruction of the breast, face, or other body part after cancer surgery or trauma. Preferably, engineered soft tissues comprises (i) a soft tissue parenchymal cell (e.g., a skin cell, subcutaneous fat cell, muscle sheath (fascia) cell, muscle cell, or adipocyte), (ii) a preadipocyte, adipocyte not having a genetic modification, perivascular cell, vascular smooth muscle cells, mesenchymal precursor cell, and fibroblast, and (iii) an endothelial cell or endothelial precursor cell. Optionally, an engineered soft tissue comprises only soft tissue parenchymal cells and preadipocytes, adipocytes having or not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, and fibroblasts. For example, a breast is generated by culturing a breast parenchymal cell, a pre-adipocyte, adipocyte, mesenchymal cell, or mesenchymal precursor cell, and an endothelial cell to produce a functional microvascular scaffold.

Parenchymal cells may be introduced to the culture, prior to, during, or after formation of the microvascular scaffold. The parenchymal cells are allowed to fill the interstitial spaces within the microvasculature.

For soft tissue reconstruction, the matrix, which is mixed with cells (e.g., (i) preadipocytes, adipocytes not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, and fibroblasts, (ii) endothelial cells, and (iii) parenchymal cells), may form a hydrogel. A hydrogel is defined as a substance formed when an organic polymer (natural or synthetic) is cross-linked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure that entraps water molecules to form a gel. Examples of materials that can be used to form a hydrogel include polysaccharides (e.g., alginate), polyphosphazenes, and polyacrylates (e.g., hydroxyethyl methacrylate). Other materials that can be used include proteins (e.g., fibrin, collagen, fibronectin) and polymers (e.g., polyvinylpyrrolidone), and hyaluronic acid.

In general, these polymers are at least partially soluble in aqueous solutions, (e.g., water) buffered salt solutions, or aqueous alcohol solutions, which have charged side groups, or monovalent ionic salts thereof. Examples of polymers with acidic side groups that can be reacted with cations are poly(phosphazenes), poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers (e.g., sulfonated polystyrene). Copolymers having acidic side groups formed by reaction of acrylic or methacrylic acid and vinyl ether monomers or polymers can also be used. Examples of acidic groups are carboxylic acid groups, sulfonic acid groups, halogenated (preferably fluorinated) alcohol groups, phenolic OH groups, and acidic OH groups.

Examples of polymers with basic side groups that can be reacted with anions are poly(vinyl amines), poly(vinyl pyridine), poly(vinyl imidazole), and some imino substituted polyphosphazenes. The ammonium or quaternary salt of the polymers can

also be formed from the backbone nitrogens or pendant imino groups. Examples of basic side groups are amino and imino groups.

Alginate can be ionically cross-linked with divalent cations in water at room temperature to form a hydrogel matrix. Additional methods for the synthesis of the other polymers described above are known to those skilled in the art (see, for example, Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, E. Goethals, editor, Pergamen Press, Elmsford, NY 1980). Many polymers, such as poly(acrylic acid), are commercially available.

Synthetic Polymers

Synthetic polymers can also be used to form a matrix, and are preferred for reproducibility and controlled release kinetics. Synthetic polymers that can be used include bioerodible polymers such as poly(lactide), poly(glycolic acid), poly(lactide-co-glycolide), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes, and non-erodible polymers such as polyacrylates, ethylene-vinyl acetate polymers and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, teflon.RTM., and nylon. Non-degradable materials can also be used to form the matrix.

One preferred non-degradable material for implantation of a matrix is a polyvinyl alcohol sponge, or alkylation or acylation derivatives thereof (e.g., ester derivatives). including esters. A non-absorbable polyvinyl alcohol sponge is available commercially as Ivalon.TM. from Unipoint Industries. These materials are all commercially available.

Preferred polymers for use in the matrix have mechanical and biochemical properties that enhance viability and proliferation of transplanted cells, tissues, or organs.

Sythetic Degradable Polymer Matrices

Synthetic degradable polymer matrices have been proposed as a means of tissue reconstruction and repair. The matrix serves as both a physical support and an adhesive substrate for isolated cells during *in vitro* culturing and subsequent *in vivo* implantation. Matrices are used to deliver cells to desired sites in the body, to define a potential space for engineered tissue, and to guide the process of tissue development. Cell transplantations on matrices are useful for the regeneration of tissues and organs (e.g., skin, nerve, liver, pancreas, cartilage and bone tissue) using various biological and synthetic materials.

Engineered Tissue and Organ Transplantation

A donor organ, donor cell, engineered tissue, or engineered organ is transplanted into a patient (e.g., a human or mammal) for the treatment or stabilization of a condition, disease, or disorder using standard methods known to the skilled artisan. For example, methods for transplanting engineered blood vessels are described by Hibino *et al.*, (Kyobu Geka 55:368-73, 2002); methods for engineered ventricular tissue transplantation are described by Krupnick *et al.*, (J. Heart Lung Transplant 21(2):233-43, 2002) and Nishina T *et al.* (Clin. Exp. Pharmacol. Physiol. 29:728-30, 2002); methods for implanting engineered skin are described by Donati *et al.*, (Biol. Neonate 80:273-6, 2001); methods for implanting a tissue-engineered stomach are described by Hori *et al.*, (ASAIO J. 47:206-10, 2001); methods for implanting an engineered bladder are described by Schoeller *et al.* (J. Urol. 165:980-5, 2001) and Oberpenning (Nat Biotechnol 17:149-55, 1999); methods for three-dimensional skeletal muscle tissue-engineering are described by Saxena *et al.*, (Biomed. Mater. Eng. 11(4):275-81, 2001); methods for adipose tissue implantation are described by Patrick (Semin. Surg. Oncol. 19:302-11, 2000; methods for implanting engineered liver support devices are described by Filipponi *et al.*, (Clin Exp Pharmacol Physiol 29:728-30, 2002) and Makowka (Surgery 88:244-253, 1980); methods for liver transplantation are described by Kalayoglu (J. Am. Coll.

Surg. 182(5):381-7, 1996); methods for implanting an engineered kidney are described by Ota *et al.*, (Laboratory & Clinical Medicine. 140(1):43-51, 2002); and methods for lung and heart transplantation are described in LeGal *et al.*, (Ann. Thorac. Surg. 49:840-4, 1990).

Additional methods of removing, storing, and transplanting an organ are standard in the art, and are described, for example, in U.S. Patent No. 5,693,462.

Methods for Evaluating Therapeutic Efficacy

Methods of the invention are useful for treating or stabilizing in a patient (e.g., a human or mammal) a condition, disease, or disorder affecting a tissue or organ. Therapeutic efficacy is optionally assayed by measuring, for example, the biological function of the treated or transplanted organ (e.g., bladder, brain, nervous tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, and uterus). Such methods are standard in the art and exemplary methods follow. Bladder function is assayed by measuring urine retention and excretion. Brain, spinal cord, or nervous tissue function is assayed by measuring neural activity (e.g., electrical activity). Esophageal function is assayed by measuring the ability of the esophagus to convey food to the stomach. Fallopian tube function is assayed by injecting radiopaque agents. Heart function is assayed by electrocardiogram. Pancreatic function is assayed by measuring insulin production. Intestinal function is assayed by measuring the ability of intestinal contents to pass through to the bowel, and may be evaluated using a barium enema or GI series. Gallbladder function is assayed using a gall bladder radionuclide scan. Kidney function is assayed by measuring creatinine levels, urine creatinine levels, or by clinical tests for creatinine clearance, or blood urea nitrogen. Liver function is assayed using liver function tests or a liver panel that measures liver enzyme levels, bilirubin levels, and albumin levels. Lung function is assayed using spirometry, lung volume, and diffusion capacity tests. Ovary function is assayed by

measuring levels of ovarian hormones (e.g., follicle stimulating hormone). Prostate abnormality is assayed by measuring prostate specific antigen. Spleen function is assayed using a technetium scan or liver-spleen scan. Stomach function is assayed using a stomach acid test or by assaying gastric emptying. Testicular function is assayed by measuring levels of testicular hormones (e.g., testosterone). Other methods for assaying organ function are known to the skilled artisan and are described, for example, in the Textbook of Medical Physiology, Tenth edition, (Guyton et al., W.B. Saunders Co., 2000).

Preferably, a transplantation method of the present invention, increases the biological function of a tissue or organ by at least 5%, 10%, 20%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, or even by as much as 300%, 400%, or 500%.

In addition, the therapeutic efficacy of the methods of the invention can optionally be assayed by measuring an increase in cell number in the treated or transplanted tissue or organ as compared to a corresponding control tissue or organ (e.g., a tissue or organ that did not receive treatment). Preferably cell number in a tissue or organ is increased by at least 5%, 10%, 20%, 40%, 60%, 80%, 100%, 150%, or 200% relative to a corresponding tissue or organ. Methods for assaying cell proliferation are known to the skilled artisan and are described in (Bonifacino *et al., Current Protocols in Cell Biology* Loose-leaf, Jossey-Bass a Wiley Co., San Francisco, CA).)

Alternatively, the therapeutic efficacy of the methods of the invention is assayed by measuring angiogenesis, blood vessel formation, or the function of a blood vessel network in the tissue or organ receiving treatment as compared to a control tissue or organ (e.g., corresponding tissue or organ that did not receive treatment). A method that increases blood vessel formation (e.g., by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 100%, 150%, or 200%, or even by as much as 300%, 400%, or 500% is considered to be useful in the invention. Methods for evaluating angiogenesis are standard in the art and are described herein.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adapt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

All publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

Claims

1. A method for inducing blood vessel formation or engineering blood vessels in a tissue or organ of a mammal, said method comprising administering one or more cells selected from the group consisting of preadipocytes, adipocytes not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, and fibroblasts to a tissue or organ of a mammal in need of increased blood vessel formation or engineered blood vessels.

2. The method of claim 1, wherein said mammal has a deficiency of at least 5% of a particular cell type.

3. The method of claim 1, wherein said mammal has damage to said tissue or organ, and wherein said administering provides a dose of cells sufficient to increase a biological function of said tissue or organ.

4. The method of claim 1, wherein said mammal has a disease, disorder, or condition, and wherein said administering provides a dose of cells sufficient to ameliorate or stabilize said disease, disorder, or condition.

5. The method of claim 1, further comprising administering to said mammal one or more cells selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, endothelial cell lines, primary culture endothelial cells, endothelial cells derived from stem cells, bone marrow derived stem cells, cord blood derived cells, HUVEC, lymphatic endothelial cells, and endothelial pregenitor cells.

6. The method of claim 1, further comprising administering a matrix to said mammal.

7. The method of claim 1, wherein said method increases the number of cells of said tissue or organ by at least 5% compared to a naturally-occurring corresponding control tissue or organ.

8. The method of claim 1, wherein said method increases the biological activity of a tissue or organ by at least 5% compared to a naturally-occurring corresponding control tissue or organ.

9. The method of claim 1, wherein said method increases blood vessel formation in said tissue or organ by at least 5% compared to a naturally-occurring corresponding control tissue or organ.

10. The method of claim 1, wherein said tissue or organ is selected from the group consisting of bladder, brain, nervous tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, and uterus, breast, skeletal muscle, skin, bone, and cartilage.

11. The method of claim 1, wherein said mammal is a human.

12. The method of claim 1, wherein said cells are part of a microvascular scaffold.

13. A method for increasing blood vessel formation or engineering blood vessels in a tissue or organ, comprising administering perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, or fibroblasts to a tissue or organ in need of increased blood vessel formation or engineered blood vessels.

14. The method of claim 13, wherein said administering to said tissue or organ is carried out *in vivo*.

15. The method of claim 13, wherein said administering to said tissue or organ is carried out *ex vivo*.

16. The method of claim 13, further comprising administering to said tissue or organ one or more cells selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, endothelial cell lines, primary culture endothelial cells, endothelial cells derived from stem cells, bone marrow derived stem cells, cord blood derived cells, HUVEC, lymphatic endothelial cells, and endothelial pregenitor cells.

17. The method of claim 13, wherein said method further comprising administering a matrix to said tissue or organ.

18. The method of claim 13, wherein said tissue or organ is selected from the group consisting of bladder, brain, nervous tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, and uterus, breast, skeletal muscle, skin, bone, and cartilage.

19. The method of claim 13, wherein said method increases the number of cells of said tissue or organ by at least 5% compared to a naturally-occurring corresponding control tissue or organ.

20. The method of claim 13, wherein said method increases the biological activity of a tissue or organ by at least 5% compared to a naturally-occurring corresponding control tissue or organ.

21. The method of claim 13, wherein said method increases blood vessel formation in said tissue or organ by at least 5% compared to a naturally-occurring corresponding control tissue or organ.

22. A method for transplanting a tissue or organ in a mammal comprising administering to said mammal a tissue or organ having at least 5% more preadipocytes, adipocytes not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, and fibroblasts than a naturally occurring control tissue or organ.

23. The method of claim 22, wherein said method further comprises administering an endothelial cell to said mammal.

24. The method of claim 23, wherein said endothelial cell is a heterologous endothelial cell.

25. The method of claim 22, wherein said method further comprises administering a matrix to said mammal.

26. A method for producing a microvascular scaffold comprising culturing (i) a first cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, endothelial cell lines, and endothelial pregenitor cells, and (ii) a second cell selected from the group consisting of preadipocytes, adipocytes, and fibroblasts, under conditions that allow formation of a microvascular scaffold.

27. A method for producing a microvascular scaffold comprising culturing (i) a first cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, endothelial cell lines, and endothelial pregenitor cells, and (ii) a second cell selected from the group consisting of perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells and mesenchymal cells, under conditions that allow formation of a microvascular scaffold.

28. The method of claim 26 or 27, wherein said first and second cells are cultured in the presence of a matrix.

29. A microvascular scaffold comprising (i) a first cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, or endothelial cell lines; and (ii) a second cell selected from the group consisting of preadipocytes, adipocytes and fibroblasts.

30. A perfused microvascular scaffold comprising (i) a first cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, and endothelial cell lines; and (ii) a second cell selected from the group consisting of perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells and mesenchymal cells.

31. The scaffold of claim 29 or 30, further comprising a matrix.

32. The scaffold of claim 31, wherein said matrix comprises a bioactive molecule.

33. The scaffold of claim 32, wherein said bioactive molecule is selected from the group consisting of activin A, adrenomedullin, aFGF, ANF, angiogenin, angiopoietin-1, angiopoietin-2, angiopoietin-3, angiopoietin-4, angiostatin, angiotropin, angiotensin-2, AtT20-ECGF, betacellulin, bFGF, B61, bFGF inducing activity, CAM-RF, ChDI, CLAF, Cox-2, ECDGF (endothelial cell-derived growth factor), ECG, ECI, EDM, EGF, EMAP, endothelins, endostatin, endothelial cell growth inhibitor, endothelial cell-viability maintaining factor, ephrins, Epo, HGF, TNF-alpha, TGF-beta, PD-ECGF, PDGF, IGF, IL8, growth hormone, fibrin fragment E, FGF-5, Factor X, HB-EGF, HBNF, HGF, HUAF, heart derived inhibitor of vascular cell proliferation, IFN-gamma, IL1, IGF-2 IFN-gamma, K-FGF, LIF, leiomyoma-derived growth factor, MCP-1, macrophage-derived growth factor, monocyte-derived growth factor, MD-ECI, MECIF, MMPs, neurothelin, nitric oxide, oncostatin M, PD-ECGF, PAI-2, PD-ECGF, PF4, PlGF, prolactin, prostacyclin, protein S, smooth muscle cell-derived growth factor, Smooth muscle cell-derived migration factor, tachykinins, TGF-beta, TIMPs, TNF-alpha, TNF-beta, transferrin, thrombospondin, urokinase, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF, VEGI, PF4, 16 kDa fragment of prolactin, prostaglandins E1 and E2, steroids, heparin, 1-butyryl glycerol (monobutyrin), and nicotinic amide.

34. The scaffold of claim 29 or 30, wherein the interstices of said scaffold further comprise a cell from the group consisting of skin cells, liver cells, heart cells, kidney cells, pancreatic cells, lung cells, bladder cells, stomach cells, intestinal cells, cells of the urogenital tract, breast cells, skeletal muscle cells, skin cells, bone cells, cartilage cells, keratinocytes, hepatocytes, gastro-intestinal cells, epithelial cells, endothelial cells, mammary cells, skeletal muscle cells, smooth muscle cells, parenchymal cells, osteoclasts, and chondrocytes.

35. A tissue comprising a scaffold of claim 29 or 30.

36. A tissue having transplanted cells of a particular cell-type and having at least 5% more cells of said cell-type than a corresponding, naturally-occuring tissue, wherein said cell-type is selected from the group consisting of preadipocytes, adipocytes not having a genetic modification, perivascular cells, vascular smooth muscle cells, mesenchymal precursor cells, mesenchymal cells, and fibroblasts.

37. The tissue of claim 36, further comprising a transplanted cell selected from the group consisting of blood vascular endothelial cells, lymph vascular endothelial cells, endothelial cell lines, primary culture endothelial cells, endothelial cells derived from stem cells, bone marrow derived stem cells, cord blood derived cells, HUVEC, lymphatic endothelial cells, and endothelial pregenitor cells.

38. The tissue of claim 35, having at least a 5% increase in blood vessel formation or engineered blood vessels after transplantion of said cells compared to a corresponding naturally-occurring tissue.

39. The tissue of claim 35, having at least a 5% increase in cell proliferation after transplantation of said cells compared to a corresponding naturally-occurring tissue.

40. The tissue of claim 35, wherein said tissue further comprises cells derived from the group consisting of bladder, brain, nervous tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, breast, skeletal muscle, skin, bone, and cartilage cells.

41. The tissue of claim 35, having at least a 5% increase in biological function after transplantation of said cells compared to a corresponding naturally-occurring corresponding tissue.

42. The tissue of claim 40, wherein said biological function is digestion, excretion of waste, secretion, electrical activity, muscle activity, hormone production, or metabolic activity.

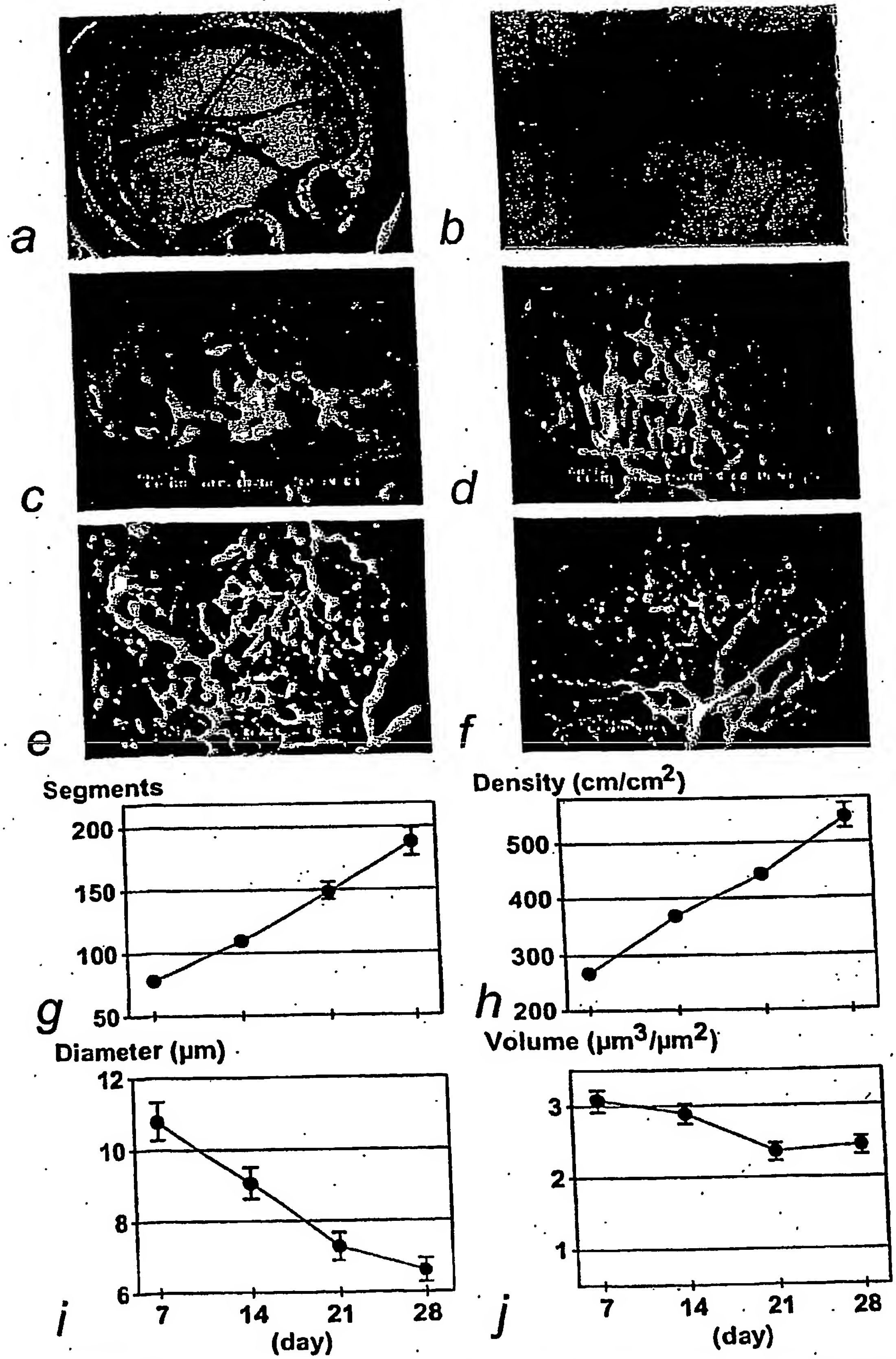
43. An organ comprising a tissue of claim 35 or 36.

REPAIRING OR REPLACING TISSUES OR ORGANS

Abstract of the Disclosure

In general, this invention provides methods and compositions for stabilizing, repairing, or replacing damaged or diseased tissues or organs by engineering blood vessels in such tissues or organs. In addition, the invention further provides methods and compositions for producing functional microvascular networks useful in tissue engineering.

Figures 1A-1J



Figures 2A-2H

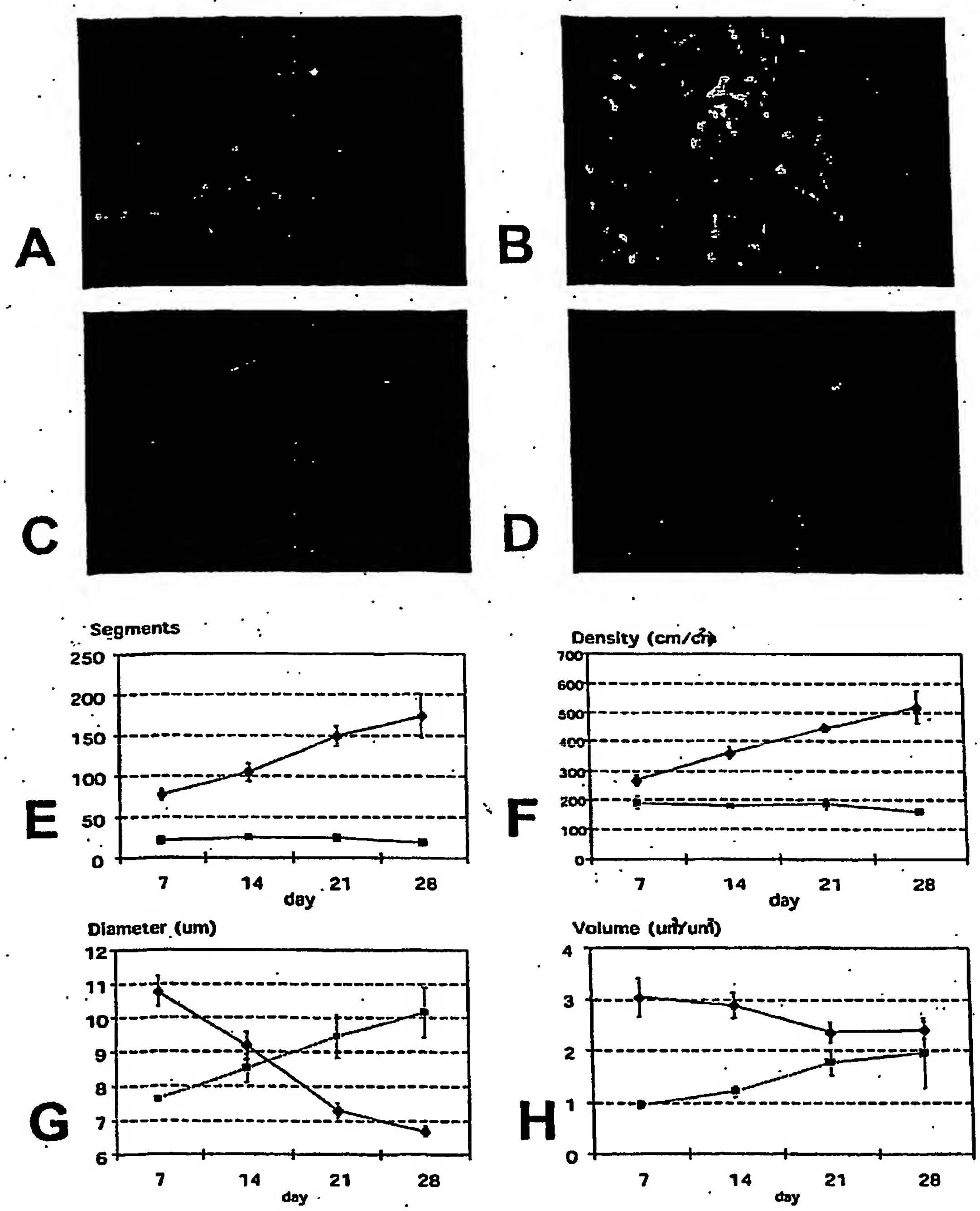
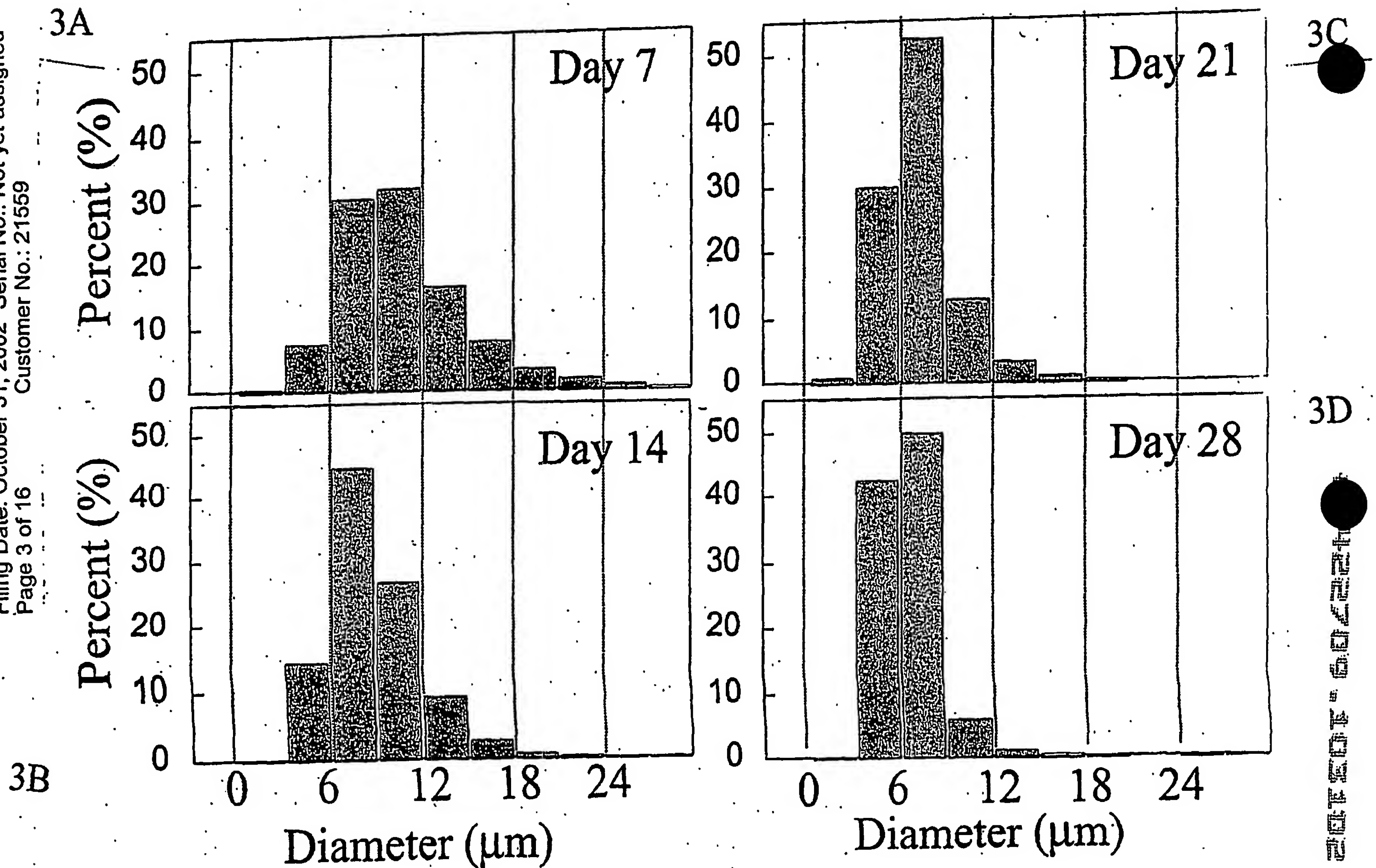


Figure 3


3A
3B
3C
3D
Day 7
Day 14
Day 21
Day 28
Percent (%)
Percent (%)
0
10
20
30
40
50
0
6
12
18
24
Diameter (µm)
Diameter (µm)

Figures 4A-4C

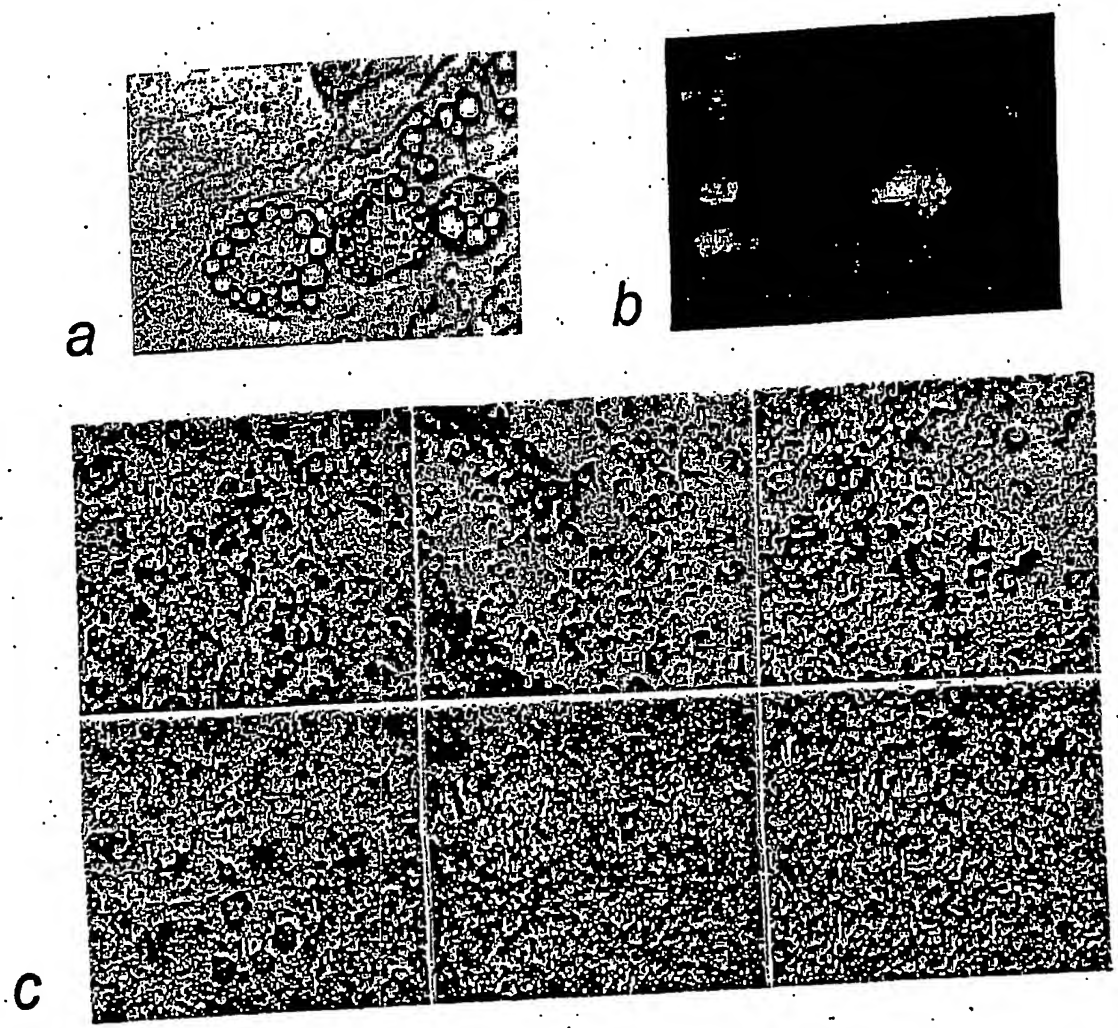


Figure 5 (page 1 of 4)

Genebank	Gene name	in vitro 3T3-442A/ PPARγ DN	3T3-442A/ Preadipocyte	3T3-442A/ Adipocyte	Comments
NM_009621	Adamts1	(+)	(+)	(+)	Decreased in adipocytes (x3)
NM_013906	Adamts8	N.D.	N.D.	N.D.	
U83509	angiopoietin-1	N.D.	N.D.	N.D.	Not detectable by RT-PCR
NM_007426	angiopoietin2	(+)	(+)	(+)	Decreased in adipocytes (x2), Increased by PPARγ DN (x27)
U22516	angiogenin	N.D.	N.D.	N.D.	
NM_007643	CD36	(+)	(+)	(+)	Increased in adipocytes (x13)
NM_009868	cadherin 5	(+)	N.D.	N.D.	
NM_007693	Vasostatin/chromogranin A	N.D.	N.D.	N.D.	
NM_009929	procollagen, type XVIII, alpha 1	N.D.	N.D.	N.D.	
M13926	G-CSF	(+)	(+)	(+)	Increased by PPARγ DN (x57)
NM_010217	Tissue factor	N.D.	N.D.	N.D.	
NM_007901	Edg1	N.D.	N.D.	N.D.	
NM_007909	Ephrin A2	N.D.	N.D.	N.D.	
NM_010109	Ephrin A receptor	N.D.	N.D.	N.D.	
NM_010111	Ephrin B2	(+)	(+)	(+)	Increased in adipocytes (x2.5)
NM_010113	EGF	N.D.	N.D.	N.D.	
NM_007912	EGFR	(+)	(+)	(+)	Decreased in adipocytes (x2.5), Increased by PPARγ DN (x5.5)
NM_007932	endoglin	N.D.	N.D.	N.D.	
NM_010144	Ephrin B4	(+)	(+)	(+)	
U71126	erb-2	N.D.	N.D.	N.D.	
NM_011808	c-ets1	N.D.	N.D.	N.D.	
NM_010168	Prothrombin kringle-1	(+)	(+)	(+)	
U67610	aFGF	N.D.	N.D.	N.D.	
NM_030614	FGF16	(+)	(+)	(+)	Increased by PPARγ DN (x2.5)
M30644	bFGF	N.D.	N.D.	N.D.	
M30642	FGF4	N.D.	N.D.	N.D.	
M92416	FGF6	N.D.	N.D.	N.D.	

Figure 5 (page 2 of 4)

U58503	FGF7/KGF	(+)	(+)	(+)	
M33760	FGFR1 (FLG)	N.D.	N.D.	N.D.	
M81342	FGFR3	N.D.	N.D.	N.D.	
NM_008011	FGFR4	(+)	(+)	(+)	Increased by PPARγ DN (x2.5)
D89628	VEGF-D/FIGF	(+)	(+)	(+)	
X59397	KDR	N.D.	N.D.	N.D.	
L07297	VEGFR	N.D.	N.D.	N.D.	
M18194	Fn1	(+)	(+)	(+)	Increased in adipocytes (x4)
J04596	Gro1	N.D.	N.D.	N.D.	
X84046	HGF	N.D.	N.D.	N.D.	
NM_010431	Hif1a	(+)	(+)	(+)	
M31885	ID1	(+)	(+)	(+)	
NM_008321	ID3	(+)	(+)	(+)	
NM_010502	IFNA1	N.D.	(+)	(+)	
NM_010510	IFN-b1	N.D.	(+)	(+)	
K00083	IFN r	(+)	N.D.	N.D.	Increased by PPARγ DN (x15)
NM_010512	IGF-1	N.D.	N.D.	N.D.	
NM_010548	IL-10	N.D.	N.D.	N.D.	
M86672	IL-12A	N.D.	N.D.	N.D.	
NM_010577	Integrin a5	(+)	(+)	(+)	
NM_008402	Integrin aV	(+)	(+)	(+)	
NM_016780	CD61	N.D.	N.D.	N.D.	
NM_008539	Madh1	(+)	(+)	(+)	
NM_010784	Midkine	(+)	(+)	(+)	
NM_008610	gelatinase A	(+)	(+)	(+)	
NM_013599	gelatinase B	N.D.	N.D.	N.D.	
NM_031195	SR-A	N.D.	N.D.	N.D.	
NM_008713	NOS3	(+)	(+)	(+)	
NM_008737	neuropilin	(+)	(+)	(+)	
M29464	PDGF a	N.D.	N.D.	N.D.	
AF162784	PDGF-b	N.D.	N.D.	N.D.	
NM_011058	PDGFRa	(+)	(+)	(+)	
NM_008809	PDGFRb	N.D.	N.D.	N.D.	

Figure 5 (page 3 of 4)

NM_008816	PECAM1	N.D.	N.D.	N.D.	
AB017491	PF 4	N.D.	N.D.	N.D.	
NM_008827	Placental growth factor	N.D.	N.D.	N.D.	
X02389	PLAU	(+)	(+)	(+)	
NM_008969	PTGS1	N.D.	N.D.	N.D.	
NM_011198	Cox-2	(+)	(+)	(+)	
D90225	pleiotrophin	(+)	(+)	(+)	
NM_019765	Restin	(+)	(+)	(+)	
NM_011333	Scya2	(+)	(+)	(+)	
NM_009257	maspin	(+)	(+)	(+)	Decreased in adipocytes (x2)
M33960	PAI-1	N.D.	N.D.	N.D.	
X16490	PAI-2	(+)	(+)	(+)	
AF017057	PEDF	(+)	(+)	(+)	
NM_009242	SPARC	(+)	(+)	(+)	Very high
NM_009263	osteopontin	N.D.	N.D.	N.D.	
D13738	Tie-2	N.D.	N.D.	N.D.	
U65016	TGF-a	N.D.	N.D.	N.D.	
M13177	TGFb1	N.D.	N.D.	N.D.	
X57413	TGF b2	N.D.	N.D.	N.D.	
M32745	TGF b3	N.D.	N.D.	N.D.	
D28526	TGFBR1(ALK-5)	N.D.	N.D.	(+)	Increased in adipocytes (x2)
NM_009371	TGFbR2	(+)	(+)	(+)	
AF039601	betaglycan	(+)	(+)	(+)	
M87276	THBS1	N.D.	N.D.	N.D.	
L07803	THBS2	(+)	(+)	(+)	
L24434	TIMP2	N.D.	N.D.	N.D.	
AF102887	THBS3	N.D.	N.D.	N.D.	
X73960	Tie1	(+)	(+)	(+)	Decreased in adipocytes (x2), Increased by PPARγ DN (x5.5)
NM_011593	TIMP1	(+)	(+)	(+)	
0	TIMP2	(+)	(+)	(+)	
NM_011607	tenascin-C	(+)	(+)	(+)	
NM_013693	TNF a	N.D.	N.D.	N.D.	
M84487	VCAM-1	N.D.	N.D.	N.D.	

Figure 5 (page 4 of 4)

M95200	VEGF	(+)	(+)	(+)	Increased in adipocytes (x2), Decreased by PPARγ DN (x)
U48800	VEGF-B	(+)	(+)	(+)	Low
U73620	VEGF-C	(+)	(+)	(+)	Low

Figures 6A-6H

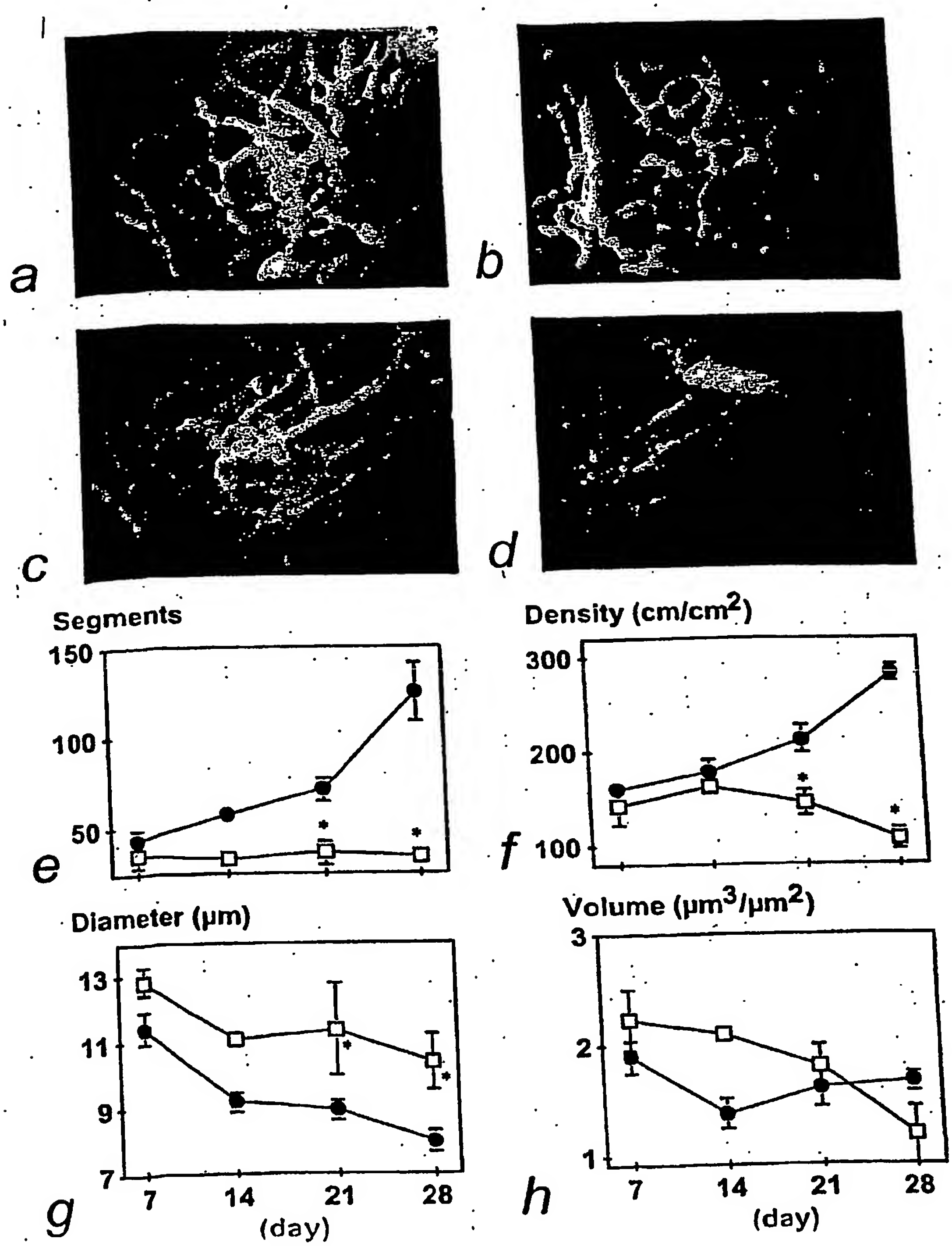


Figure 7

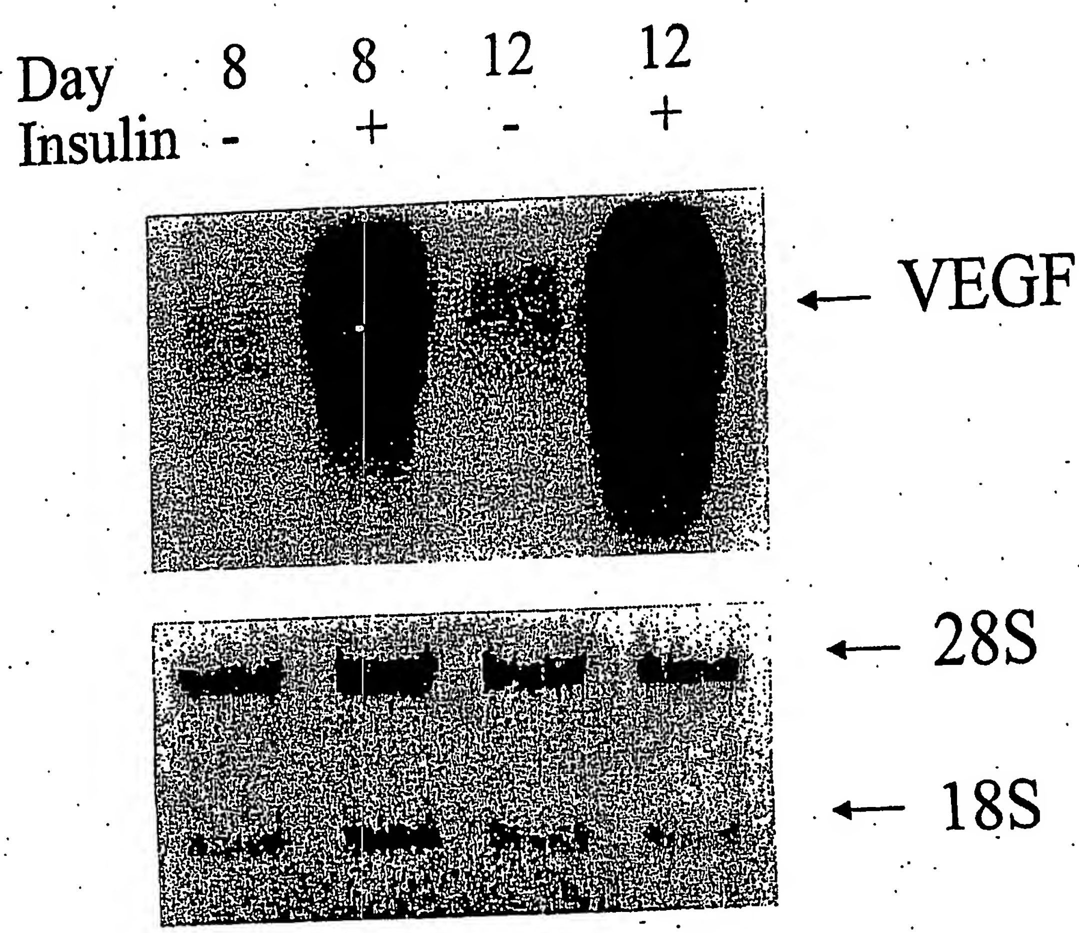


Figure 8

Gene name	Sense primer	Antisense primer
Ang-1	5'-ATGACAGTTTTCCTTTCCTTTGCA-3' (SEQ ID NO: 1) & 5'-TTCTTCGCTGCCATTCTGACTCAC-3' (SEQ ID NO: 2)	5'-CATCATGGTGGTGGAACGTAAGGA-3' (SEQ ID NO: 3)
Ang-2	5'-ATGTGGCAGATCATTTTCCTAACT-3' (SEQ ID NO: 4) & 5'-TTTGGCTGGGATCTTGTCTTGGC-3' (SEQ ID NO: 5)	5'-CCAGTAGTACCACTTGATACCGTT-3' (SEQ ID NO: 6)
aP2	5'-CTGGAAGACAGCTCCTCCTCGAAG-3' (SEQ ID NO: 7) & 5'-ATGTGTGATGCCTTTGTGGGAAC-3' (SEQ ID NO: 8)	5'-TAATCAACATAACCATATCCAAT-3' (SEQ ID NO: 9)
VEGF	5'-TCC GGA TCC ATG AAC TTT C-3' (SEQ ID NO: 10)	5'-TGG CTC ACC GCC TTG GCT-3' (SEQ ID NO: 11)
L13a	5'-GGGGCAGGTTCTGGTATTGGATG-3' (SEQ ID NO: 12)	5'-CCTCGCACAGTGCGCCAGAAAATG-3' (SEQ ID NO: 13)
VEGF-B	5'-GCTGCTTGTTGCACTGCTGCAGCT-3' (SEQ ID NO: 14)	5'-TCTGCATTCACATTGGCTGTGTTC-3' (SEQ ID NO: 15)
VEGF-C	5'-ATGCACTTGCTGTGCTTCTTGTCT-3' (SEQ ID NO: 16)	5'-CTCTGTGTTATAATGTGCAGCAGC-3' (SEQ ID NO: 17)
VEGF-D	5'-CCTCATGATGTTCCATGTGTACTT-3' (SEQ ID NO: 18)	5'-TGTCTCTCTAGGGCTGCATTGGGT-3' (SEQ ID NO: 19)
Osteopontin	5'-CATGAGATTGGCAGTGATTTGCTT-3' (SEQ ID NO: 20)	5'-TCTGCTTGTGTACTAGCAGTGACG-3' (SEQ ID NO: 21)
Efrin B2	5'-AGGGACTCTGTGTGGAAGTACTGTT-3' (SEQ ID NO: 22)	5'-CTGGTCTGGCACAGTTGAGCAGTG-3' (SEQ ID NO: 23)
HIF1α	5'-GGCGGCGAGAACGAGAAGAAAAAG-3' (SEQ ID NO: 24)	5'-CACGTTATCAGAAATGTAAACCAT-3' (SEQ ID NO: 25)

Figure 9A

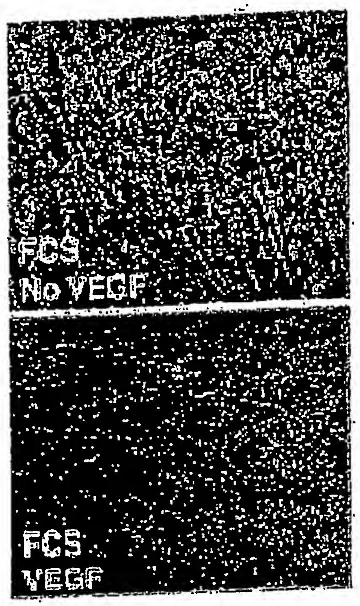
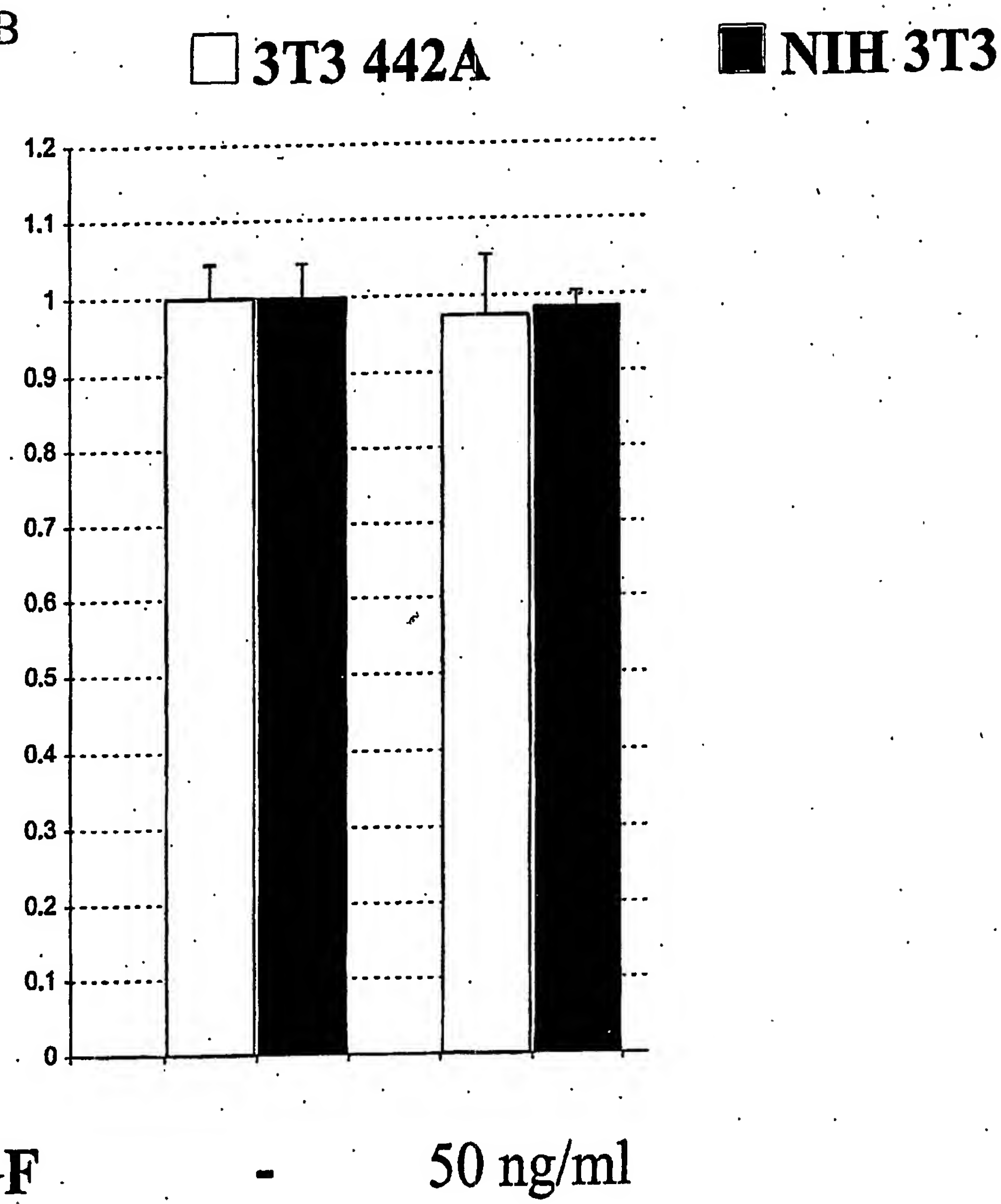
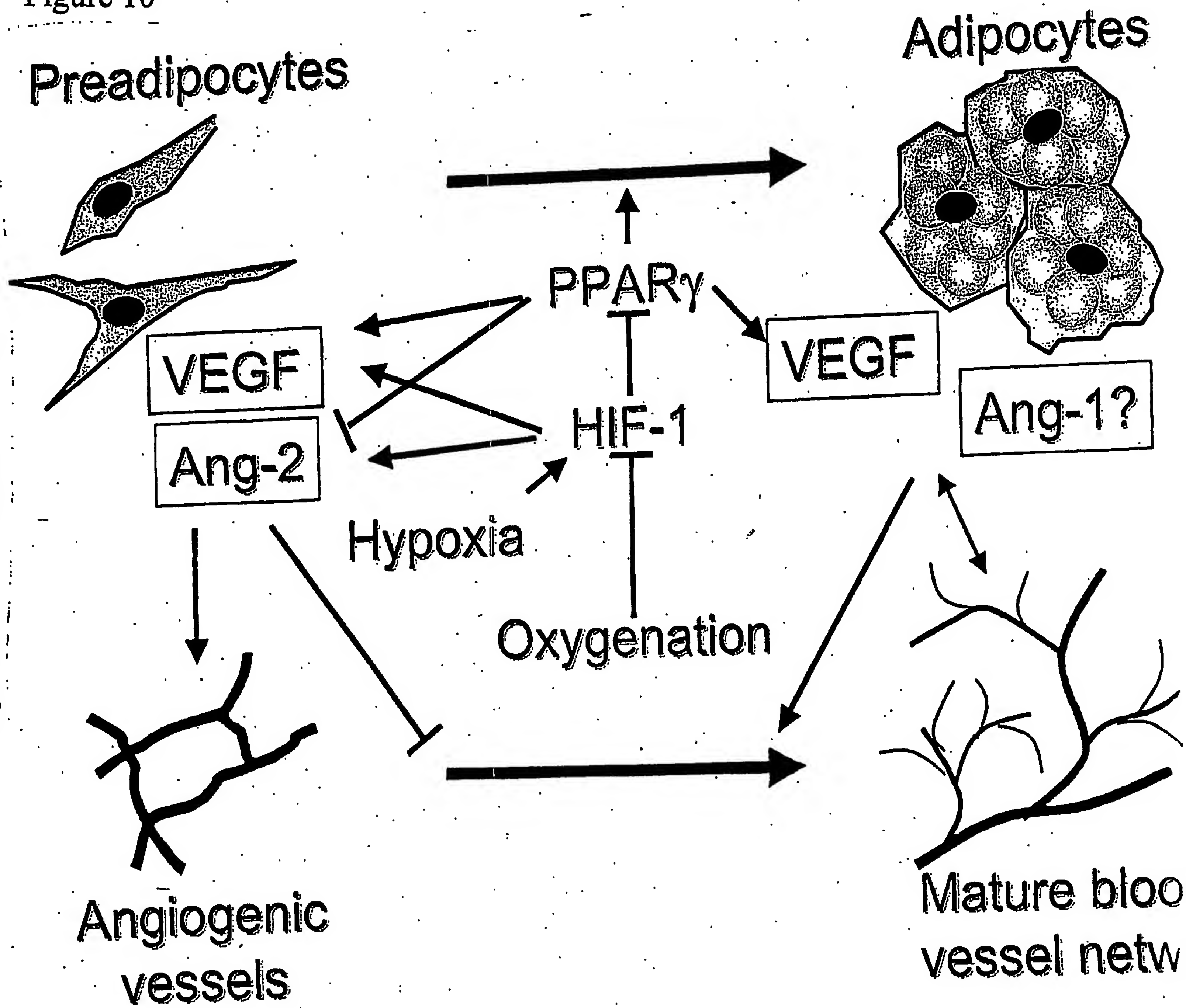


Figure 9B



Title: Repairing or Replacing Tissues or Organs
Applicant(s): Rakesh K. Jain et al.
Filing Date: October 31, 2002 Serial No.: Not yet assigned.
Page 14 of 16 Customer No.: 21559

Figure 10



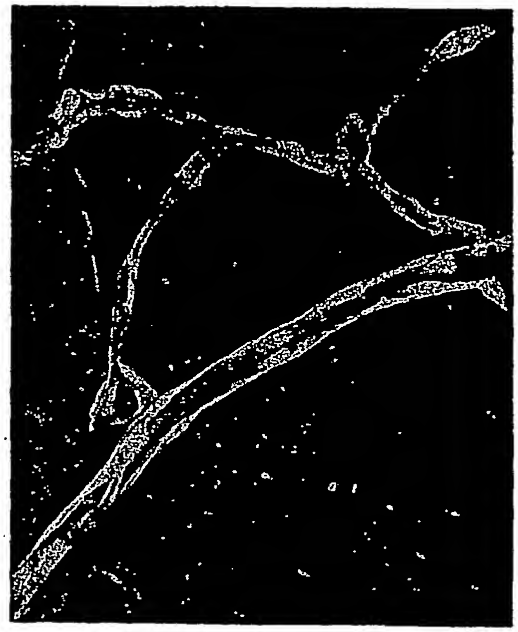


Figure 11

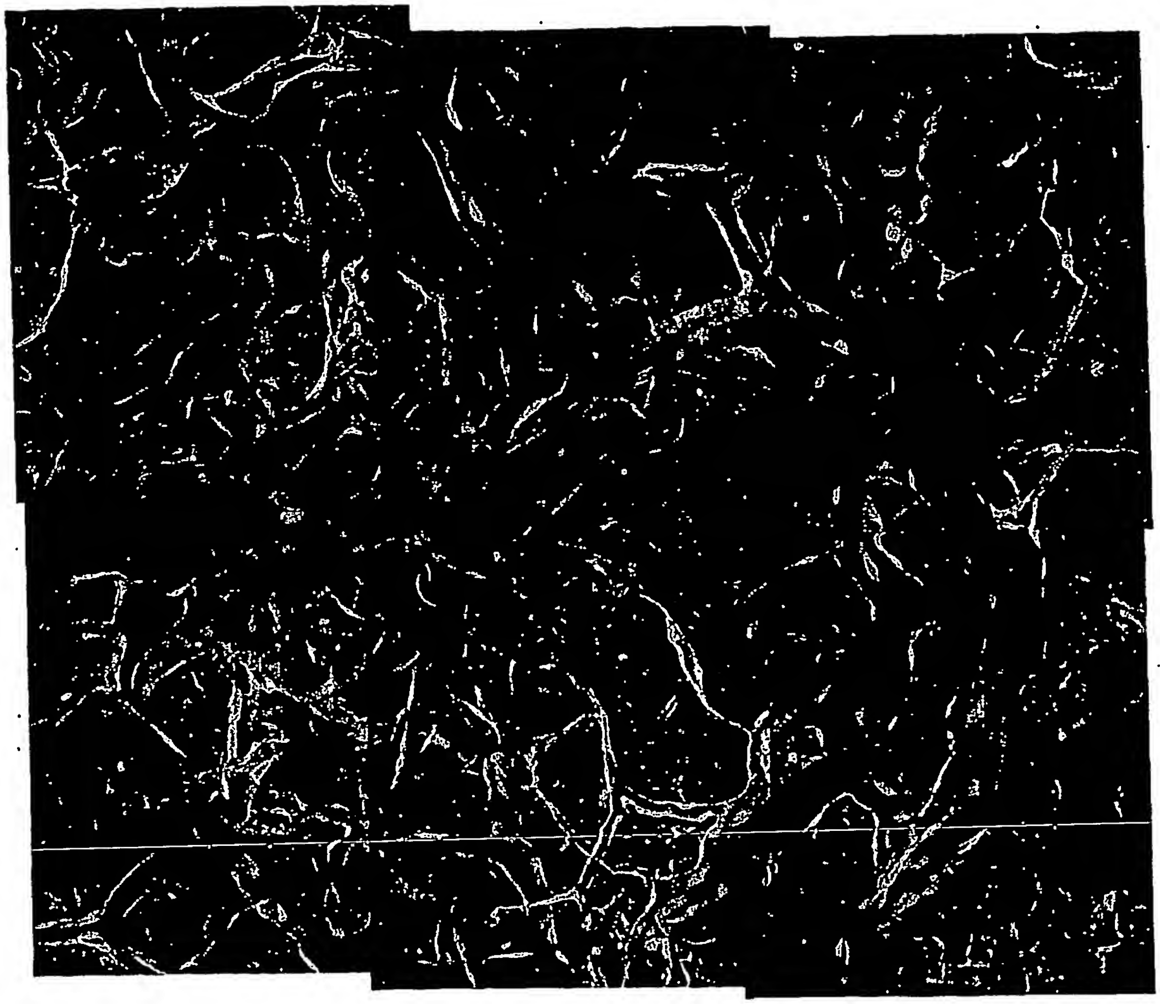


Figure 12